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(54) Title: METHOD OF DETECTION AND INTERPRETATION OF MUTATIONS THROUGH EXPRESSION OR FUNCTION TESTS OF HAPLOID GENES

(57) Abstract: The present invention relates to a method for detection and interpretation of loss-of-function or gain-of-function mutations for test genes of interest. The genes of interest include those associated with inherited genetic disorders. The present invention involves the process of obtaining a sample of genetic material from an individual in the form of tissue or cells, separation of the genetic material from the cells of the individuals into haploid sets by transferring the individual chromosomal entities into a population of target cells, and monitoring the target cell population for successful transfer and expression of the test genes of interest using various functional, immunological and structural assays.

METHOD OF DETECTION AND INTERPRETATION OF MUTATIONS THROUGH EXPRESSION OR FUNCTION TESTS OF HAPLOID GENES

SPECIFICATION

FIELD OF THE INVENTION

The present invention relates to a method for detection and interpretation of disease related mutations through the combination of haploid gene transfer with functional, immunological or other analysis of the gene product.

BACKGROUND OF INVENTION

Detection of disease-causing mutations is a complex and challenging task in medical and veterinary genetics and research. Unfortunately, loss-of-function mutations, including partial loss-of-function mutation, or gain-of-function mutations, including alteration of function and dominant negative mutations, causing inherited genetic diseases are a common problem for humans and other animals. Complete and effective detection of these mutations presents enormous possibilities as a diagnostic, preventative, or research tool.

Currently genomic sequencing of peripheral blood DNA is widely used for identification of genetic mutations associated with various diseases. In particular, it may be used to detect mutations in individuals for inherited genetic diseases. For example, Myriad Genetics, Inc. (Salt Lake City, UT) has developed a genetic test for detection of loss-of-function mutations in BRCA1 and BRCA2, genes which have been linked to breast cancer. This test sequences all coding exons of BRCA1 and BRCA2, making it labor-intensive and costly. In addition, it cannot detect deleted exons, inversions, mutations causing loss of transcriptional activity, etc. As a result, many mutations in these two genes cannot be meaningfully detected by genomic sequencing. Table 1 displays the types and frequencies of mutations found in the BRCA1 and BRCA2 genes. Furthermore, when diploid cells that are heterozygous for a loss-of-function or a gain-of-function mutation are tested, the wild type allele can often mask the mutant allele. As a result, this test may not be accurate in

detecting single mutant alleles. The usefulness of this and other such tests to the medical and veterinary professions and research scientists is therefore limited by their diagnostic shortcomings and prohibitive costs.

Table 1: Frequency and Type of Mutations in the BRCA1 and BRCA2 Genes

Mutation Type	BRCA1 Gene	BRCA2 Gene
Frameshift	195 (42.5%)	126 (53.5%)
Nonsense	55 (12%)	20 (7.8%)
Splice	16 (3.5%)	4 (1.6%)
Missense	21 (4.6%)	12 (4.7%)
Large Deletion	3 (0.7%)	-
Polymorphism	37 (8%)	6 (2.4%)
Yet Unclassified	132 (28.7%)	76 (30%)
Total Number	459 (100%)	254 (100%)

The Protein Truncation Test (PTT) is another diagnostic test available for the detection of loss-of-function alleles, which involves *in vitro* transcription and translation of the gene of interest, followed by gel electrophoretic analysis. This test is designed to detect mutations that produce a truncated protein. While this test provides an efficient means of detecting nonsense mutations, it is of no real use for detection of many other common mutations, such as frameshift, missense, inversions, and other mutations that have no detectable effect on the size of the transcribed protein.

Microarrays present another means of detecting mutations. In these assays thousands of specific oligonucleotides complementary to all known base substitutions, insertions and deletions for a gene of interest are bound to glass slides. Fluorescently labeled PCR-amplified fragments from the gene of interest are then hybridized to the microarray and binding to a particular oligonucleotide is detected. Microarrays have high up-front costs and are also not accurate at detecting heterozygous mutations. They are further limited to detection of mutations represented in the oligonucleotides.

A number of indirect methods for molecular detection of mutations exist. These include single-strand conformation polymorphism, denaturing gradient gel electrophoresis, denaturing high-performance liquid chromatography and other electrophoretic or enzymatic-based methods. Each of these methods is limited in the types of mutations it can detect and in its ability to detect heterozygous mutations in general.

To overcome the difficulty in the detection of heterozygote genotypes for inherited genetic disorders, Yan., "Conversion of diploidy to haploidy", *Nature 403*: 723-724 (February, 2000) (Yan (1)), Yan *et al.*, "Genetic testing-Present and Future", *Science 298*: 1890-1891 (September, 2000) (Yan (2)), and Zoghbi *et al.*, "Assignment of Autosomal Dominant Spinocerebellar Ataxia (SCA1) Centromeric to the HLA Region on the Short Arm of Chromosome 6, Using Multilocus Linkage Analysis", *Am. J. Hum. Genet. 44*: 255-263 (1989) have all proposed a method of genetic testing using somatic cell hybrids haploid for a chromosome of interest. This method manipulates the two copies (alleles) of a gene of interest from a donor cell by separating the two chromosomes so that each can be analyzed individually. Detection of heterozygous mutations by these methods is improved in such cells because the wild type allele has been eliminated and cannot mask the mutated allele. However, the method described requires extremely labor intensive and impractical techniques for the isolation and segregation of haploid hybrids bearing the desired chromosome in a haploid state. Further, while the nucleic acid analysis of the haploid cells would facilitate detection of exon deletions, inversions, and transcriptional defects, the approach does not offer a significant advantage over traditional methods. Yan (2)

admit that "[i]t is important to note that Conversion [the Yan *et al.* approach] is not a substitute for the [traditional] detection methods described above, but rather is an adjunct that provides improved nucleic acid templates that can maximize the sensitivity of conventional methods", *Science 289*, p.1892. Yan (2) further admit that "[d]isadvantages of the Conversion [Yan *et al.*] approach include the increased time and expense associated with the hybrid generation and screening process", *Science 289*, p.1892. Thus, while the proposed method offers an improvement over the conventional screening methods, reliance on the conventional methods is not abolished and the improvement in detection is slight, especially in light of the dramatic increases in time and expense associated with the method.

Several other methods of transferring one or multiple chromosomes to a host cell have been previously described (U.S. Pat. No. 4,806,476; WO 00/34436; U.S. Pat. No. 6,077,697). This method, microcell-mediated chromosome transfer (MMCT) was first described by Fournier and Ruddle for the transfer of murine chromosomes from one cell to another (PNAS 74: 319-323 (1977)) and by McNeill and Brown for the transfer of single human chromosomes from one cell to another (PNAS 77:5394-5398 1980). MMCT describes a way of generating microcells, by prolonged colcemid and cytochalsin B treatment of donor cells, which contain one or more chromosomes or chromosomal fragments from donor cells, and fusing them using polyethylene glycol (PEG) with target cells to generate microcell hybrids, haploid for the desired chromosome/chromosomal fragment from the donor cell (Figure 2). While these papers presents an efficient means of generating haploid cells, they fail to describe a method employing easily obtainable donor cells. In the paper of Fournier and Ruddle, mouse embryo fibroblasts were used as donors for microcell-mediated chromosome transfer. McNeill and Brown utilized human foreskin fibroblasts as donors for human chromosome transfer.

Therefore, there is a need for a medically, veterinarily, or scientifically useful method of detecting loss-of-function mutations, including partial loss-of-function mutations, or gain-of-function mutations, including alteration of function and dominant negative mutations, in any of a variety of genes. The present invention addresses the deficiencies of the prior art by providing a method for genetic

testing using easily obtainable sources of genetic material that can 1) detect many types of mutations, including nonsense, missense, frameshift, deletions, inversions, etc. , 2) easily detect heterozygous and homozygous mutations, and 3) less time-consuming, labor-intensive and cheaper than known methods of genetic testing.

SUMMARY OF THE INVENTION

The present invention relates to a method for detection and interpretation of loss-of-function or gain-of-function mutations for test genes of interest. The present invention involves the process of obtaining a sample of genetic material from an individual in the form of tissue or cells, separation of the genetic material from the cells of the individuals into haploid sets by transferring the individual chromosomal entities into a population of target cells, and monitoring the target cell population for successful transfer and expression of the test genes of interest using various functional, immunological and structural assays (Figure 1). Preferably, the test gene or genes of interest are associated with known inherited human and animal disorders.

In an embodiment of the invention, the sample of genetic material from an individual with a potential genetic abnormality is in the form of cells or tissue sample. The donor cells from the individual may be any cell type obtained from the individual. In another embodiment of the invention, the individual would provide a blood sample containing peripheral blood cells. In a further embodiment of the invention, donor cells may be lymphoblasts prepared from the individual's blood.

The genetic material comprising the test gene or genes may be located on naked DNA, plasmid, chromosome or chromosomal fragments. In a preferred embodiment of the invention, the test gene is located on a chromosome or chromosomal fragment.

In an embodiment of the invention, the separation of genetic material from donor cells into haploid sets by transfer to a population of target cells can be accomplished using various known methods of gene transfer. In a preferred embodiment of the invention, microcell mediated cell transfer (MMCT) is used to transfer genetic material to target cells.

In a preferred embodiment of the invention, the target cells may be any cell which is capable of accepting genetic material from donor cells, retaining it as a stable entity and expressing the test gene product. In a preferred embodiment of the invention, the test gene product is expressed at detectable levels. Expression of the test gene may occur through endogenous cell machinery or through cellular and molecular manipulation of cells.

In an embodiment of the invention, the presence of the test gene or genes are monitored in the target cells. In a preferred embodiment of the invention, the test gene product is monitored in the target cells. In a most preferred embodiment of the invention, the test protein is monitored. Immunofluorescence may be employed to detect test protein of interest.

In an embodiment of the invention, presence of the test gene or genes is detected by fluorescence *in situ* hybridization (FISH) or chromosomal painting. In yet another embodiment of the invention, the presence of the test gene is detected by fluorescent-activated cell sorting (FACS) analysis.

In another embodiment of the invention, the test gene or genes are detected though the use of a relevant functional assay for test protein function. This assay is designed based on knowledge of the cellular, immunological, molecular, biochemical, physiological, genetic, structural characteristics of the test gene product or products of interest. It takes into account all relevant functional information to design an appropriate functional assay. Assays which may be employed include, but are limited to, immunofluorescence, FACS, two-hybrid inhibition assay, ion channel activity, mismatch repair assay, and endocytic uptake of labeled LDL (low density lipoprotein).

In another embodiment of the invention, the presence of the test gene is monitored through the presence of a closely linked gene. The target cells may be monitored for either presence of linked gene or gene product, by fluorescence in situ hybridization (FISH), chromosomal painting, or fluorescent-activated cell sorting (FACS) analysis. In a preferred embodiment of the invention, known surface protein markers from specific chromosomes shared by the test gene may be used as the

closely linked gene. The use of a relevant functional assay may also be employed to detect the presence of a closely linked gene and its gene products.

In another embodiment of the invention, the genotype of the donor individual may be determined by evaluating the ratio of the number of cells expressing the wild type gene product to the number of cells expressing the test gene product.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the general steps of the method of the present invention when used to detect a loss-of-function mutation.

Figure 2 shows MMCT as described by Killary *et al.*

Figure 3 shows an assay that may be used to detect the gene product of a gene linked to the test gene. Detection of a linked gene indicate which target cells have received the target gene.

Figure 4 depicts a potential assay for loss-of-function mutations in mismatch repair-related test genes, such as those associated with HNPCC, using mismatch repair deficient target cells.

Figure 5 shows an inhibition two-hybrid inhibition assay that may be used to detect functional test protein.

Figure 6 depicts an *in vivo* assay that may be used to detect functional test protein.

Figure 7 shows two embodiments of the invention for gain-of-function mutations.

Figure 8 shows several possible embodiments of the invention.

Figure 9 shows an embodiment of the invention for more than one test gene.

Figure 10 is a FACS profile of CHO cells that have taken up Human Chromosome 19 and demonstrating efficient LDL uptake. The left graph indicates inside the ellipse the cell population from which later information was drawn. The middle graph shows FL1-control fluorescence. The right graph shows FL2-dil-LDL fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

Figure 11 shows fluorescence in CHO cells incubated with dil-LDL 20% of which did receive Human Chromosome 19 and 80 % of which did not receive Human Chromosome 19 evaluated through LDL uptake. The left graph indicates inside the ellipse the cell population from which later information was drawn. The middle graph shows FL1-control fluorescence. The right graph shows FL2-dil-LDL fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

Figure 12 shows a variation of MMCT used in the preferred embodiment in which LDLR is the test gene.

Figure 13 (a) shows expression of ICAM-1 in mouse L cells through FACS. The upper left graph indicates inside the ellipse the cell population from which later information was drawn. The lower left graph shows FL2-control fluorescence. The lower right graph shows FL1-FITC-anti-ICAM-1 fluorescence. The upper right graph plots FL2-control fluorescence v. FL1-FITC-anti-ICAM-1 fluorescence. Measurements for the areas marked in the graphs are shown in the chart. Figure 16(b) shows that ICAM-1 negative mouse L cells are also distinguishable by FACS. The upper left graph indicates inside the ellipse the cell population from which later information was drawn. The lower left graph shows FL2-control fluorescence. The lower right graph shows FL1-FITC-anti-ICAM-1 fluorescence. The upper right graph plots FL2-control fluorescence v. FL1-FITC-anti-ICAM-1 fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

Figure 14 shows the FACS analysis detection of ICAM-1 or LDLR in a somatic cell hybrid that originally contained Human Chromosome 19, but in which some cells have undergone spontaneous loss of the chromosome. The upper left graph indicates inside the ellipse the cell population from which later information was drawn. The lower left graph shows FL2-dil-LDL fluorescence. The lower right graph shows FL1-FITC-anti-ICAM-1 fluorescence. The upper right graph plots FL2-dil-LDL fluorescence v. FL1-FITC-anti-ICAM-1 fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

Figure 14 (a) shows the results of FACS analysis for ICAM-1 and LDLR for a normal individual. Figure 14 (b) shows the results of FACS analysis for ICAM-1 and LDLR for another normal individual. In both figures, the upper left graph indicates inside the ellipse the cell population from which later information was drawn. The middle left graph shows FL2-dil-LDL fluorescence. The upper right graph shows FL1-FITC-anti-ICAM-1 fluorescence. The upper middle graph plots FL2-dil-LDL fluorescence v. FL1-FITC-anti-ICAM-1 fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

Figure 16 (a) shows the result of FACS analysis for ICAM-1 and LDLR for an individual heterozygous for a loss-of-function mutation in the LDLR gene. Figure 16 (b) shows the result of FACS analysis for ICAM-1 and LDLR for another individual heterozygous for a loss-of-function mutation in the LDLR gene. In both figures, the upper left graph indicates inside the ellipse the cell population from which later information was drawn. The middle left graph shows FL2-dil-LDL fluorescence. The upper right graph shows FL1-FITC-anti-ICAM-1 fluorescence. The upper middle graph plots FL2-dil-LDL fluorescence v. FL1-FITC-anti-ICAM-1 fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

Figure 17 (a) shows the result of FACS analysis for ICAM-1 and LDLR for an individual homozygous for a loss-of-function mutation in the LDLR gene. Figure 17 (b) shows the result of FACS analysis for ICAM-1 and LDLR for another individual homozygous for a loss-of-function mutation in the LDLR gene. In both figures, the upper left graph indicates inside the ellipse the cell population from which later information was drawn. The middle left graph shows FL2-dil-LDL fluorescence. The upper right graph shows FL1-FITC-anti-ICAM-1 fluorescence. The upper middle graph plots FL2-dil-LDL fluorescence v. FL1-FITC-anti-ICAM-1 fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

Figure 18 (a) shows the result of FACS analysis for ICAM-1 and LDLR for control cells. Figure 18 (b) shows the result of FACS analysis for ICAM-1 and LDLR for another set of control cells. In both figures, the upper left graph

indicates inside the ellipse the cell population from which later information was drawn. The middle left graph shows FL2-dil-LDL fluorescence. The upper right graph shows FL1-FITC-anti-ICAM-1 fluorescence. The upper middle graph plots FL2-dil-LDL fluorescence v. FL1-FITC-anti-ICAM-1 fluorescence. Measurements for the areas marked in the graphs are shown in the chart.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for detecting and interpreting loss-of-function or gain-of-function mutations in a variety of genes. Loss-of-function and gain-of-function mutations, including dominant negative mutations, cause many known diseases and disorders in humans, including breast and ovarian cancer, familial hypercholesterolemia, hereditary nonpolyposis colon cancer (HNPCC), neurofibromatosis, polyposis of the colon, Duchenne dystrophy, cystic fibrosis, Li Fraumeni disease, tuberous sclerosis, Gorlin syndrome, Von Hippel-Lindau disease, porphyrias, osteogenesis imperfecta, Marfan's disease, polycystic kidney disease, hemophilia, SCID, Rett syndrome, lysosomal diseases, and ornithine transcarbamylase (OTC) deficiency. Detection of loss-of-function and gain-of-function mutations that can result in these and other diseases may be useful for *inter alia*, laboratory research, medical diagnosis leading to proper counseling and treatment of those afflicted with the diseases at both the pre and post natal stages of development, and genetic testing for potential carriers of various diseases. The techniques described may also be used to detect loss-of-function or gain-of-function mutations in animals. Humans are not alone in our affliction with diseases resulting from such mutations; animal research, veterinary science and practice, and animal husbandry will also benefit by detecting loss-of-function and gain-of-function mutations in animals and employing that knowledge to better treat and breed animals.

The present invention provides a method of detecting loss-of-function or gain-of-function mutations in individuals homozygous or heterozygous for a genetic abnormality by separating the two chromosomes from an individual donor cell so that each copy of the gene from the chromosome can be analyzed individually. The analysis requires the genetic transfer of each chromosome to a target cell population to produce somatic cell hybrids. This allows for haploid analysis of each

chromosomal entity. Separation of the two copies of the gene facilitate the detection of heterozygous mutations. The wild type alleles can often mask the effect of the mutant allele in various methods of genetic testing. Individual cells from the hybrid populations can then be scored for presence or absence of the test gene of interest. Figure 1 shows a schematic of the invention to detect a heterozygous loss-of-function mutation.

The term "test gene" as used here and throughout the specification may refer to the traditional concept of a gene or the gene and its flanking syntenic DNA. The amount of genetic material designated as the "test gene" will vary depending on the location and type of mutation to be detected. For instance, the "test gene" may encompass a large flanking region if a deletion is to be detected. Furthermore, the test gene product may be referred to as the test protein through the specification and claims, as this is the gene product that will most commonly be examined with this method. However, one skilled in the art will appreciate that the test gene product may encompass nucleic acids and protein molecules.

Though the term test gene is used in the above summary and throughout the specification and claims, it will be understood to one skilled in the art that in other embodiments of the invention more than one test gene may be transferred to from the donor cell to the target cell. The target cells may be assayed for the presence of the each test gene separately, or, if the test genes are linked, one assay may confirm the presence of all test genes. Further, different functional or immunological assays may be performed to detect functional or wild type protein expression for each test gene separately or, if the genes function in concert, a single assay that requires functional or wild type expression of each gene may be employed.

Source of Donor Cells

To practice the invention, a sample of genetic material which is collected from an individual with a known potential genetic abnormality is collected as a cell or tissue sample. Blood is a common source of genetic material used for genetic testing. Lymphoblasts are an potential important source of cultured cells and may also be obtained from blood. A mitogen, such as phytohemagglutanin, can be used to induce lymphoblasts from peripheral blood cells. Cells may be obtained from

any bodily fluids or tissues, including tissue from biopsies. Other somatic and gamete cells may also be used. Cells such as lymphoblasts and sperm cells have the advantage of being easily obtainable. However, any cell type or mixture of cell types is appropriate, provided that the cells may be obtained in sufficient quantities to allow transfer of the test gene from donor to target cells.

Genetic Transfer

In an embodiment of the invention, the test gene of interest is detected in a hybrid target cell population after genetic transfer to allow for haploid analysis of each copy of the test gene. The transfer of the test gene or genes is accomplished through transfer of genetic material comprising the test gene or genes.

Numerous mechanisms for transferring a gene from one cell to another are known to the art. Any such mechanism presently known or later developed is suitable for the transfer of the test gene so long as the mechanism results in the transfer of only one copy of the test gene to substantially all target cells or a distinguishable portion of target cells that receive the test gene. The transfer may be performed by means yet to be discovered or by mechanisms known to one skilled in the art. Such mechanisms include microcell-mediated chromosome transfer (MMCT), electroporation, liposome-mediated gene transfer, somatic cell fusion, gamete cell fusion, injection of gamete cells into target cells, biolistic transfer and other known transfection protocols. See Killary, A., *et al.*, "Functional Studies to Identify Tumor Suppressor Genes", *Methods: A Companion to Methods in Embryology 8*: 234-246 (1995); Yan, H. ., "Conversion of Diploidy to Haploidy", *Nature 403*, 723-724 (February 17, 2000); WO 00/34436, "FACS Assisted Methods for Introducing Individual Chromosomes Into Cells" to Nolan, E. *et al.* (June 15, 2000); U.S. Pat. No. 6,077,697, "Artificial Chromosomes, Uses Thereof and Methods for Preparing Artificial Chromosomes", to Hadlaczky, G. *et al.* (June 20, 2000); U.S. Pat. No. 4,806,476, "Efficient Cell Fusion Process" to Coons, T. *et al.* (1989); Aslam, I., *et al.*, "Evaluation of the fertilization potential of freshly isolated, in-vitro cultured and cryopreserved human spermatids by injection into hamster oocytes", *Hum. Reprod. 14*: 1528 (1999); U.S. Pat. No. 4,806,476; WO 00/34436; U.S. Pat No. 6,077,692; Yan *et al.* (2000); Johnston, S.A. and Tang, D.C., "The use of

microparticle injection to introduce genes into animal cells *in vitro* and *in vivo*", *Genet. Eng. (N.Y.) 15*: 225-236 (1993); Sanford, J.C., *et al.*, "Optimizing the biolistic process for different biological applications", *Methods Enzymol. 217*: 483-509 (1993), incorporated herein by reference, for examples of some potential transfer methods.

To practice the invention, many techniques for gene transfer may be applied as indicated above. These mechanisms can apply since the test gene may be a located on an excised piece of native DNA, on a plasmid, on a chromosome or chromosomal fragment. Depending on the desired comprehensiveness of the assay, the test gene may comprise the entire native gene with most regulatory elements or it may comprise fewer elements down to a portion of an exon artificially located in a construct that will allow its expression in the target cells. In a preferred embodiment of the invention, use of the entire gene and its regulatory elements, as will be possible with MMCT, will encompass a greater range of possible loss-of-function or gain-of-function mutations. However, for many research, diagnostic and other purposes, only mutations in a portion of the gene and its regulatory elements may be of interest.

Target Cells

To practice the invention, the target cell must be carefully chosen to ensure that cells are susceptible to desired methods for gene transfer. The cells must be amenable to DNA transfer techniques described above. These target cells may include but not be limited to the following primary and transformed cell lines, mammalian, murine, insect, yeast cells and Chinese Hamster ovary (CHO) cells.

The target cells must also be chosen to ensure optimal detection of test gene or test gene products. In a preferred embodiment of the invention, the presence of the test gene is evaluated through detection of the test gene products. As a result, the target cell must possess gene-specific machinery required for expression of test gene. For detection of a test gene that is ubiquitously expressed, nearly any cell type may serve as target cells. Some ubiquitously expressed genes that may be assayed by the present invention include those encoding the low density lipoprotein receptor (LDLR) (associated with familial hypercholsterolemia) , BRCA1, BRCA2 (associated with breast and ovarian cancer), NF1, NF2 (associated with neurofibromatosis), APC

(associated with polyposis of the colon), and various genes associated with hereditary nonpolyposis colon cancer. Test genes which are not ubiquitously expressed will require a target cell that can express the test gene. This may involve using a tissue-specific cell type that can endogenously express the gene product or manipulation of the target cells to achieve expression of the test gene in the hybrid cell population. One skilled in the art will be aware of various cell, molecular, immunological, biochemical, pharmacological methods commonly used in the art to produce such a cell line.

In a preferred embodiment of the invention, the presence of the test gene will be evaluated through detection of the translated test protein. In this scenario, the target cell must be chosen to provide an environment in which the test protein expression or test protein function of the test gene can be assayed. In particular, the target cell should not express orthologs or other proteins that may interfere with the chosen assays for expression or function of the test protein. To overcome problems resulting from interfering proteins expressed endogenously by the target cell, one may apply various cell, molecular, immunological, biochemical, pharmacological methods commonly used in the art to prevent this expression. For example, the target cell may be induced to inhibit expression of ortholog or other protein. In addition, one may alter the assay in order to only detect the test protein of interest. An antibody that does not cross react with the endogenous protein may be used.

In addition, one may choose a target cell that lacks the ortholog or other protein which interferes with the detection assay. These cells may be either a genetically or functional knockout cell line. For example, Chinese Hamster Ovary (CHO) cells are appropriate for assays involving the low density lipoprotein receptor (LDLR), since CHO cells do not express LDLR, but have been shown capable of expressing the fully funtional protein in Corsetti, *et al.* (1991). Such a cell line would then allow functional analysis of the test gene product. Examples of such potential target cells include human or mouse cell lines with homozygous mutations leading to complete deficiency of the human or mouse BRCA1 or BRCA2 gene product. Other examples would be human or rodent cell lines genetically deficient for mismatch

repair enzymes such as MSH2, MLH1, PMS1 or PMS2, such that the target cells could be analyzed for function of a human mismatch repair gene. Such analysis might comprise testing the target cells for microsatellite instability using a test system introduced after gene transfer. As mentioned above, yeast cells have great potential as target cells because of the presence of many mutant strains. They are additionally valuable because of the ease with which deficiencies may be induced.

Detection of Target Gene Transfer

Following the successful transfer of the test gene from the donor cells to the target cells, it becomes necessary to detect the presence of absence of the gene in the target cells. This can be accomplished in multiple ways and the optimal strategy depends on the method for overall analysis of the cells. The assay need only detect that transfer of the test gene occurred. It need not detect the number of copies of the test gene, as only transfer methods that rarely result in transfer of more than one copy of the test gene to any target cell are suitable for this method.

Detection of DNA or Chromosome

FISH (fluorescence in situ hybridization) may be used to determine the presence or absence of a copy of the test gene in a target cell using various cloned DNA fragments such as YACs, BACs, or PACs and the standard techniques. FISH may be accomplished by many methods depending on the test gene and cell types, but some possible methods may be found in the Savage, J.R. and Tucker, J.D., "Nomenclature systems for FISH-painted chromosome aberrations", *Mutat. Res. 366(2)*: 153-156 (1996) and Bickmore, W., "Fluorescence in situ hybridization of chromosome and chromatin structure", *Methods Enzymol. 304*: 650-662 (1999), incorporated herein by reference. FISH is most suitable for analysis on microscope slides, but it may be possible to use this approach in combination with fluorescence activated cell sorting (FACS).

Chromosome paint is another alternative for detecting the presence or absence of the test gene. Chromosome paint may be performed as described in Lichter, P. *et al.*, "Detection of chromosomal aberrations by means of molecular cytogenetics: painting of chromosomes and chromosomal subregions and comparative

genomic hybridization", *Methods in Enzymology 254*: 334-359 (1995); and Tucker, J.D., *et al.*, "PAINT: A proposed nomenclature for structural aberrations detected by whole chromosome painting", *Mutat. Res. 347(1)*: 21-24 (1995), incorporated herein by reference. If a method that transfers most or all of a chromosome, such as MMCT, is used, chromosome paint detection may be efficient. Although the chromosome may become fragmented in a small number of the cells, this should not interfere with the analysis. Like FISH, chromosome paint is most suitable for analysis on microscopic slides. However, chromosome paint may also be coupled with FACS for easier detection and would prove more effective with FACS than would FISH with a single copy probe.

Detection of Protein Gene Product

In a most preferred embodiment, the hybrid target cell population is evaluated for presence or absence of the test gene by detection of protein gene product. The functional protein may be detected by immunological analysis designed to detect primarily functional or wild type proteins. Commercial antibodies are available for various epitopes of the protein. Thus, one may test with one or more antibodies to assess the presence of the protein. The antibodies used for detection must be able to distinguish the mutant test protein from the wild type functional protein. In the scenario of a test gene that expresses a missense mutation, an antibody which recognizes an epitope specific to the full length protein may be used. An immunological test could be performed as immunostaining of target cells on slides or with FACS. Analysis could also be performed using western blots to detect any protein products of abnormal size. One example of immunological detection is provided in Marcus, V.A., *et al.*, "Immunohistochemistry for hMLH1 and hMLH2: a practical test for DNA mismatch repair-deficient tumors", *Am. J. Surg. Pathol. 23*: 1248 (1999), incorporated by reference herein..

Linked Genes

In another embodiment of the invention, the presence of the test gene is monitored through the presence of a closely linked gene. For some test genes, an assay for a closely linked gene may prove to be a more efficient means of detecting

target cells that have received the test gene. (See Figure 3 for a basic description of such an assay.) Use of this analysis requires that the form of genetic material and method of transfer employed allow transfer of the linked gene to the target cell. Transfer of a chromosome or chromosome fragment by MMCT is an appropriate method for genetic transfer of a test gene and its linked gene. The linked gene should be chosen so that recombination or chromosomal degradation are unlikely to result in its separation from the test gene before or after transfer to the target cell.

This type of analysis has the added advantage of excluding any cells that received the test gene, but are incapable of expressing the test gene product properly for reasons unrelated to the allele present. However, when employing this type of assay one must take care to choose target cells capable of expressing the linked gene product and that do not express orthologs or other protein which may interfere with a functional assay.

The detection of the linked gene may occur through fluorescence in situ hybridization (FISH), or chromosome paint methods. Further, FISH and chromosome paint methods may also be coupled with FACS (fluorescence activated cell sorting) to separate target cells that have received the test gene from those that have not.

Similarly, the detection of the linked gene may occur through detection of the translated protein. Immunodetection, FACS analysis and functional assay may be applied to locate the subset of the target population that expresses the translated protein for the linked gene. The linked gene may also encode a surface protein, which can be detected through immunodetection or FACS analysis.

Known surface antigens and their human chromosome location are listed in Tables 2-25. Each of these surface antigens, as well as those not listed and not yet discovered may serve as an appropriate linked genes in an assay to determine whether a particular test gene has been received by the target cell.

Table 2: Surface Antigens of Human Chromosome 1

Long Name	Abbreviation
MEMBRANE COMPONENT, CHROMOSOME 1, SURFACE MARKER 1	M1S1
PHOSPHODIESTERASE I/NCLEOTIDE PYROPHOSPHATES 1	PDNP1
FLOTILLIN 2	FLOT2
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
TRANSMEMBRANE 4 SUPERFAMILY, MEMBER 1	TM4SF1
HEMOGLOBIN--BETA LOCUS	HBB
LETHAL ANTIGEN--A1	AL-A1
HEMOPHILIA A	
WILMS TUMOR 1	WT1
ATP-BINDING CASSETTE, SUBFAMILY B, MEMBER 2	ABCB2
MEMBRANE COMPONENT, CHROMOSOME 4, SURFACE MARKER 1	M4S1
LEUKOCYTE ADHESION DEFICIENCY TYPE I	LAD
THY-1 T-CELL ANTIGEN	THY1
ATAXIA-TELANGIECTASIA	AT
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
INTEGRIN, BETA-3	ITGB3
CD80 ANTIGEN	CD80
T-LYMPHOCYTE SURFACE CD2 ANTIGEN	CD2
RETINOBLASTOMA	RB1

RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
KANGAI 1	KAI1
PROTEIN-TYROSINE PHOSPHATASE, NONRECEPTOR TYPE, SUBSTRATE 1	PTPNS1
THYMOCYTE ANTIGEN CD1A	CD1A
CHEDIAK-HIGASHI SYNDROME	CHS1
HEMOCHROMATOSIS	HFE
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
T-LYMPHOCYTE SURFACE ANTIGEN LY-9	LY9
FACTOR V DEFICIENCY	
ALZHEIMER DISEASE	AD
INTEGRIN, BETA-2	ITGB2
ANTITHROMBIN III DEFICIENCY	
BULLOUS PEMPHIGOID ANTIGEN 1	BPAG1
SELECTIN	SELL
TUMOR PROTEIN p53	TP53
SOLUTE CARRIER FAMILY 3, MEMBER 1	SCLC3A1
PROTEIN-TYROSINE PHOSPHATASE, RECEPTOR-TYPE, C	PTPRC
INTEGRIN, BETA-1	ITGB1
HEPATOCELLULAR CARCINOMA	

PREGNANCY-SPECIFIC BETA-1-GLYCOPROTEIN 2	PSG2
INTEGRIN, ALPHA-L	ITGAL
TUMOR NECROSIS FACTOR LIGAND SUPERFAMILY, MEMBER 6	TNFSF6
DECAY-ACCELERATING FACTOR FOR COMPLEMENT	DAF
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 4	TNFRSF4
AGGRECAN 1	AGC1
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A

Table 3: Surface Antigens of Human Chromosome 2

Long Name	Abbreviation
MEMBRANE COMPONENT, CHROMOSOME 4, SURFACE MARKER 1	M4S1
HEMOGLOBIN--BETA LOCUS	HBB
HEMOPHILIA A	
FLOTILLIN 2	FLOT2
MEMBRANE COMPONENT, CHROMOSOME 17, SURFACE MARKER 2	M17S2
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
ATAXIA-TELANGIECTASIA	AT
WISKOTT-ALDRICH SYNDROME	WAS
RETINOBLASTOMA	RBI
DIPEPTIDYLPEPTIDASE IV	DPP4
INTEGRIN, BETA-2	ITGB2
CD86 ANTIGEN	CD86

HEMOCHROMATOSIS	HFE
INTEGRIN, ALPHA-2	ITGA2
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
WILMS TUMOR 1	WT1
CD8 ANTIGEN, ALPHA POLYPEPTIDE	CD8A
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
BETA-2MICROGLOBULIN	B2M
INTERLEUKIN 2 RECEPTOR, ALPHA	IL2RA
ALZHEIMER DISEASE	AD
MAJOR HISTOCOMPATABILITY COMPLEX, CLASS I, A	HLA-A
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
TUMOR PROTEIN p53	TP53
MEMBRANE COMPONENT, CHROMOSOME 6, POLYPEPTIDE 2	M6P2
ANTIGEN DEFINED BY MONOCLONAL ANTIBODY F10.44.2	
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
ADENOSINE DEAMINASE	ADA
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 2	MS4A2
PREGNANCY-SPECIFIC BETA-1-GLYCOPROTEIN 2	PSG2
ATP-BINDING CASSETTE, SUBFAMILY B, MEMBER 2	ABCB2
VON WILLEBRAND DISEASE	
INSULIN-LIKE GROWTH FACTOR 2 RECEPTOR	IGF2R

BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
ANTIGEN CD28	CD28
LEUKOCYTE ADHESION DEFICIENCY, TYPE 1	LAD
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
MEMBRANE COMPONENT, CHROMOSOME 1, SURFACE MARKER 1	M1S1
LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN-ASSOCIATED PROTEIN	LRPAP1
INTEGRIN, BETA-3	ITGB3
SOLUTE CARRIER FAMILY 3, MEMBER 1	SLC3A1
IMMUNODEFICIENCY, PARTIAL COMBINED, WITH ABSENCE OF HLA DETERMINANTS AND BETA-2-MICROGLOBULIN FROM LYMPHOCYTES	
ZETA-CHAIN-ASSOCIATED PROTEIN KINASE	ZAP70
IMMUNODEFICIENCY WITH HYPER-IgM	
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
ANTITHROMBIN III DEFICIENCY	
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
INTERLEUKIN 2 RECEPTOR, BETA	IL2RB
ANTIGEN DEFINED BY MONOCLONAL ANTIBODY TRA-2-10	M1C10

Table 4: Surface Antigens of Human Chromosome 3

Long Name	**Abbreviation**
TRANSMEMBRANE 4 SUPERFAMILY, MEMBER 1	TM4SF1
HEMOGLOBIN--BETA LOCUS	HBB

ATAXIA-TELANGIECTASIA	AT
HEMOPHILIA A	
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
INTEGRIN, BETA-3	ITGB3
MUSCULAR DYSTROPHY, PPSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
IMMUNODEFICIENCY WITH HYPER-IgM	
RETINOBLASTOMA	RB1
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
FUCOSYLTRANSFERASE 4	FUT4
WILMS TUMOR 1	WT1
CD80 ANTIGEN	CD80
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
TRANSFERRIN	TF
HEMOCHROMATOSIS	HFE
CD47 ANTIGEN	CD47
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS 1, A	HLA-A
TRANSFERRIN RECEPTOR	TFRC
DISACCHARIDE INTOLERANCE 1	
CD86 ANTIGEN	CD86
DESMOGLEIN 3	DSG3
LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN, TYPE 3	LFA3
MELANOMA-ASSOCIATED ANTIGEN p97	MF12

TUMOR PROTEIN p53	TP53
ALZHEIMER DISEASE	AD
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
VON WILLEBRAND DISEASE	
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
SOLUTE CARRIER FAMILY 3, MEMBER 1	SLC3A1
MEMBRANE METALLOENDOPEPTIDASE	MME
WISKOTT-ALDRICH SYNDROME	WAS
LEUKOCYTE ADHESION DEFICIENCY, TYPE 1	LAD
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
FLAUJEAC FACTOR DEFICIENCY	
CD151 ANTIGEN	CD151
ADENOSINE DEAMINASE	ADA
INTEGRIN, ALPHA-M	ITGAM
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
T-LYMPHOCYTE SURFACE CD2 ANTIGEN	CD2
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
ANTITHROMBIN III DEFICIENCY	
CHEDIAK-HIGASHI SYNDROME	CHS1
FACTOR V DEFICIENCY	
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
GOLGI AUTOANTIGEN, GOLGIN SUBFAMILY A, 4	GOLGA4

SIALYLTRANSFERASE 1	SIAT1
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
INTEGRIN, BETA-2	ITGB2

Table 5: Surface Antigens of Human Chromosome 4

Long Name	Abbreviation
MEMBRANE COMPONENT, CHROMOSOME 4, SURFACE MARKER 1	M4S1
TRANSMEMBRANE 4 SUPERFAMILY, MEMBER 1	TM4SF1
INTEGRIN, BETA-3	ITGB3
HEMOPHILIA A	
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
HEMOGLOBIN--BETA LOCUS	HBB
FUCOSYLTRANSFERASE 4	FUT4
ATAXIA-TELANGIECTASIA	AT
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
BLOOD GROUP--MN LOCUS	MN
RETINOBLASTOMA	RB1
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
WILMS TUMOR 1	WT1
INTERLEUKIN 4 RECEPTOR	*IL4R*
ALZHEIMER DISEASE	AD

HEMOCHROMATOSIS	HFE
ANTIGEN CD38 OF ACUTE LYMPHOBLASTIC LEUKEMIA CELLS	CD38
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 2	MS4A2
CENTROMERIC PROTEIN C1	CENPC1
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 1	MS4A1
TUMOR PROTEIN p53	TP53
SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 6	STAT6
LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN-ASSOCIATED PROTEIN 1	LRPAP1
WISKOTT-ALDRICH SYNDROME	WAS
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 4	TNFRSF4
TUMOR NECROSIS FACTOR LIGAND SUPERFAMILY, MEMBER 4	TNFSF4
BLOOD GROUP--Ss LOCUS	Ss
IMMUNODEFICIENCY WITH HYPER-IgM	
MEMBRANE COMPONENT, CHROMOSOME 1, SURFACE MARKER 1	M1S1
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
VON WILLEBRAND DISEASE	
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
FACTOR V DEFICIENCY	
NEURITE OUTGROWTH INHIBITOR	
GOGLI AUTOANTIGEN, GOLGIN SUBFAMILY A, 4	GOLGA4

ADENOSINE DEAMINASE	ADA
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
CHEDIAK-HIGASHI SYNDROME	CHS1
ANTITHROMBIN III DEFICIENCY	
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
INTEGRIN, BETA-2	ITGB2
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFSRSF6
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
CD59 ANTIGEN P18-20	CD59
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA

Table 6: Surface Antigens of Human Chromosome 5

Long Name	**Abbreviation**
HEMOGLOBIN--BETA LOCUS	HBB
HEMOPHILIA A	
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
IMMUNODEFICIENCY WITH HYPER-IgM	
ATAXIA-TELANGIECTASIA	AT
RETINOBLASTOMA	RB1
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR

HEMOCHROMATOSIS	HFE
INTEGRIN, ALPHA-2	ITGA2
WILMS TUMOR 1	WT1
MONOCYTE DIFFERENTIATION ANTIGEN CD 14	CD14
TUMOR PROTEIN p53	TP53
SURFACE ANTIGEN 5	S5
ALZHEIMER DISEASE	AD
LEUKOCYTE ANTIGEN GROUP FIVE	LAG5
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
ADENOSINE DEAMINASE	ADA
ANTIGEN MSK39 IDENTIFIED BY MONOCLONAL ANTIBODY 5.1H11	MSK39
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 5	TNFRSF5
LYMPHOCYTE ANTIGEN 64, RADIOPROTECTIVE, 105-KD	LY64
WISKOTT-ALDRICH SYNDROME	WAS
VON WILLEBRAND DISEASE	
RHESUS BLOOD GROUP CcEe ANTIGENS	RHCE
SOLUTE CARRIER FAMILY 7, MEMBER 5	SLC7A5
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
T-LYMPHOCYTE SURFACE CD2 ANTIGEN	CD2
CHEDIAK-HIGASHI SYNDROME	CHS1
INTEGRIN, BETA-3	ITGB3
PROTEIN-TYROSINE PHOSPHATASE, RECEPTOR-TYPE, C	PTPRC

SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
FLOTILLIN 2	FLOT2
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
BLOOD GROUP--MN LOCUS	MN
BULLOUS PEMPHIGOID ANTIGEN 1	BPAG1
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTOR, TWO DOMAINS, SHORT CYTOPLASMIC TAIL, 2	KIR2DS2
MEMBRANE COMPONENT, CHROMOSOME 17, SURFACE MARKER 2	M17S2
TYROSINE HYDROXYLASE	TH
FACTOR V DEFICIENCY	
L1 CELL ADHESION MOLECULE	L1CAM
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
HYPOPHOSPHATEMIA, X-LINKED	
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
ANTITHROMBIN III DEFICIENCY	
BLOOD GROUP--LUTHERAN SYSTEM	LU

Table 7: Surface Antigens of Human Chromosome 6

Long Name	Abbreviation
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
HEMOCHROMATOSIS	HFE
HEMOPHILIA A	
MEMBRANE COMPONENT, CHROMOSOME 6,POLYPEPTIDE 2	M6P2
HEMOGLOBIN--BETA LOCUS	HBB
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
KANGAI 1	DAII1
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
SURFACE ANTIGEN 6	S6
INSULIN-LIKE GROWTH FACTOR 2 RECEPTOR	IGF2R
BULLOUS PEMPHIGOID ANTIGEN 1	BPAG1
ATAXIA-TELANGIECTASIA	AT
CD59 ANTIGEN P18-20	CD59
CD83 ANTIGEN	CD83
ATP-BINDING CASSETTE, SUBFAMILY B, MEMBER 2	ABCB2
RETINOBLASTOMA	RB1
CD24 ANTIGEN	CD24
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR

WILMS TUMOR 1	WT1
SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 6	STAT6
TUMOR PROTEIN p53	TP53
RH-NULL, REGULATOR TYPE	RHN
TUMOR NECROSIS FACTOR LIGAND SUPERFAMILY, MEMBER 6	TNFSF6
RHESUS BLOOD GROUP-ASSOCIATED GLYCOPROTEIN	RHAG
WISKOTT-ALDRICH SYNDROME	WAS
LYMPHOCYTE ANTIGEN 6 COMPLEX, LOCUS E	LY6E
ALZHEIMER DISEASE	AD
INTEGRIN, BETA-3	ITGB3
TAP-BINDING PROTEIN	TAPP
LYMPHOCYTE ANTIGEN 6 COMPLEX, LOCUS H	LY6H
ADENOSINE DEAMINASE	ADA
SIALYLTRANSFERASE 1	SIAT1
VON WILLEBRAND DISEASE	
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
IMMUNODEFICIENCY WITH HYPER-IgM	
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
RHESUS BLOOD GROUP, CcEe	RHCE
FACTOR V DEFICIENCY	

CD9 ANTIGEN	CD9
ANTITHROMBIN III DEFICIENCY	
INTEGRIN, BETA-2	ITGB2
TROPHOBLAST GLYCOPROTEIN	TPBG, M6P1
DIABETES INSIPIDUS, NEPHROGENIC, X-LIKED	
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
L1 CELL ADHESION MOLECULE	L1CAM

Table 8: Surface Antigens of Human Chromosome 7

Long Name	Abbreviation
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
HEMOPHILIA A	
HEMOGLOBIN--BETA LOCUS	HBB
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
HEPATOCELLULAR CARCINOMA	
CD36 ANTIGEN	CD36
RETINOBLASTOMA	RB1
ATAXIA-TELANGIECTASIA	AT
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	

BLOOD GROUP-KELL-CELLANO SYSTEM	KEL
HEMOCHROMATOSIS	HFE
TUMOR PROTEIN p53	TP53
WILMS TUMOR 1	WT1
ALZHEIMER DISEASE	AD
HOMEO BOX GENE HB9	HLXB9
AMPHIPHYSIN	AMPH
SIX-TRANSMEMBRANE EPITHELIAL ANTIGEN OF THE PROSTATE	STEAP
SOLUTE CARRIER FAMILY 7, MEMBER 5	SLC7A5
WISKOTT-ALDRICH SYNDROME	WAS
ADENOSINE DEAMINASE	ADA
VON WILLEBRAND DISEASE	
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
FACTOR DEFICIENCY	
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
LEUKOCYTE ADHESION DEFICIENCY, TYPE 1	LAD
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
SURFACE ANTIGEN 6	S6
LETHAL ANTIGEN--ALL-A1	
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG

CHEDIAK-HIGASHI SYNDROME	CHS1
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
IMMUNODEFICIENCY WITH HYPER-IgM	
TYROSINE HYDROXYLASE	TH
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
ANTITHROMBIN III DEFICIENCY	
L1 CELL ADHESION MOLECULE	L1CAM
MEMBRANE COMPONENT, CHROMOSOME 1, SURFACE MARKER 1	M1S1
INTERLEUKIN 4 RECEPTOR	IL4R
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 1	MS4A1
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
INTEGRIN, ALPHA-E	ITGAE
AGGRECAN 1	AGC1

Table 9: Surface Antigens of Human Chromosome 8

Long Name	Abbreviation
HEMOPHILIA A	
HEMOGLOBIN--BETA LOCUS	HBB

PLASMINOGEN ACTIVATOR, TISSUE	PLAT
ATAXIA-TELANGIECTASIA	AT
HEPATOCELLULAR CARCINOMA	
WILMS TUMOR 1	WT1
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
RETINOBLASTOMA	RB1
HEMOCHROMATOSIS	HFE
A DISINTEGRIN AND METALLOPROTEINASE DOMAIN 8	ADAM8
TUMOR PROTEIN p53	TP53
LYMPHOCYTE ANTIGEN 6 COMPLEX, LOCUS E	LY6E
ALZHEIMER DISEASE	AD
SURFACE ANTIGEN 8	S8
INTEGRIN, BETA-3	ITGB3
WISKOTT-ALDRICH SYNDROME	WAS
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
VON WILLEBRAND DISEASE	
LYMPHOCYTE ANTIGEN 6 COMPLEX, LOCUS H	LY6H
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
FACTOR V DEFICIENCY	
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
ADENOSINE DEAMINASE	ADA

THROMBASTHENIA OF GLANZMANN AND NAEGELI	
TUMOR NECROSIS FACTOR LIGAND SUPERFAMILY, MEMBER 8	TNFSF8
CD59 ANTIGEN P18-20	CD59
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLCA4A1
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6,	TNFRSF6
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
IMMUNODEFICIENCY WITH HYPER-IgM	
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
CD44 ANTIGEN	CD44
L1 CELL ADHESION MOLECULE	L1CAM
ANTITHROMBIN III DEFICIENCY	
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
INTEGRIN, BETA-2	ITGB2
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
CD8 ANTIGEN, ALPHA POLYPEPTIDE	CD8A
SURFACE ANTIGEN MIC2	MIC2
THYMOCYTE ANTIGEN CD1A	CD1A
LETHAL ANTIGEN--A1	ALL-A1
TYROSINE HYDROXYLASE	TH
CD9 ANTIGEN	CD9

DIPEPTIDYLPEPTIDASE IV	DPP4
BETA-2 MICROGLOBULIN	B2M
XG BLOOD GROUP SYSTEM	XG; PBDX
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE

Table 10: Surface Antigens of Human Chromosome 9

Long Name	Abbreviation
T-LYMPHOCYTE SURFACE sLY-9	LY9
HEMOPHILIA A	
WILMS TUMOR 1	WT1
HEMOGLOBIN-BETA LOCUS	HBB
ATAXIA-TELANGIECTASIA	AT
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
RETINOBLASTOMA	RB1
ALZHEIMER DISEASE	AD
HEMOCHROMATOSIS	HFE
TUMOR PROTEIN p53	TP53
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
INTEGRIN, BETA-3	ITGB3
VON WILLEBRAND DISEASE	

THROMBASTHENIA OF GLANZMANN AND NAEGELI	
ADENOSINE DEAMINASE	ADA
CD59 ANTIGEN P18-20	CD59
WISKOTT-ALDRICH SYNDROME	WAS
TUMOR NECROSIS FACTOR LIGAND SUPERFAMILY, MEMBER 8	TNFSF8
MEMBRANE COMPONENT, CHROMOSOME 4, SURFACE MARKER 1	M4S1
IMMUNODEFICIENCY WITH HYPER-IgM	
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
FACTOR V DEFICIENCY	
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
MEMBRANE COMPONENT, CHROMOSOME 1, SURFACE MARKER 1	M1S1
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
CHEDIAK-HIGASHI SYNDROME	CHS1
THY-1 T-CELL ANTIGEN	THY1
T-LYMPHOCYTE SURFACE CD2 ANTIGEN	CD2
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
CD9 ANTIGEN	CD9

INTERLEUKIN 2 RECEPTOR, ALPHA	IL2RA
XG BLOOD GROUP SYSTEM	XG; PBDX
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
DIPEPTIDYLPEPTIDASE IV	DPP4
TRANSFERRIN RECEPTOR	TFRC
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
BULLOUS PEMPHIGOID ANTIGEN 1	BPAG1
TRANSFERRIN	TF
ANTITHROMBIN III DEFICIENCY	
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
L1 CELL ADHESION MOLECULE	L1CAM
HYPOPHOSPHATEMIA, X-LINKED	

Table 11: Surface Antigens of Human Chromosome 10

Long Name	**Abbreviation**
HEMOPHILIA A	A
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
INTERLEUKIN 2 RECEPTOR, ALPHA	IL2RA
INTEGRIN, BETA-1	RB1
RETINOBLASTOMA	RB1
HEMOGLOBIN--BETA LOCUS	HBB

MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
ATAXIA-TELANGIECTASIA	AT
HEMOCHROMATOSIS	HFE
WILMS TUMOR 1	WT1
A DISINTEGRIN AND METALLOPROTEINASE DOMAIN 8	ADAM8
CD39 ANTIGEN	CD39
TUMOR PROTEIN p53	TP53
WISKOTT-ALDRICH SYNDROME	WAS
ALZHEIMER DISEASE	AD
ANTIGEN DEFINED BY MONOCLONAL ANTIBODY TRA-2-10	MIC10
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
NEUROPILIN 1	NRP1
ADENOSINE DEAMINASE	ADA
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
VON WILLEBRAND DISEASE	
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
ANTITHROMBIN III DEFICIENCY	
IMMUNODEFICIENCY WITH HYPER-IgM	

FACTOR V DEFICIENCY	
INTEGRIN, BETA-3	ITGB3
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
THY-1 T-CELL ANTIGEN	THY1
CD44 ANTIGEN	CD44
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
PLASMINOGEN ACTIVATOR, TISSUE	PLAT
CD59 ANTIGEN P18-20	CD59
T-CELL ANTIGEN RECEPTOR, DELTA SUBUNIT	TCRD
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
INTEGRIN, BETA-2	ITGB2
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
MEMBRANE COMPONENT, CHROMOSOME 1, SURFACE MARKER 1	M1SI
LEUKOCYTE ADHESION DEFICIENCY, TYPE 1	LAD
XG BLOOD GROUP SYSTEM	SG; PBDX
THYMOCYTE ANTIGEN CD1A	CD1A
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
CD36 ANTIGEN	CD36
SURFACE ANTIGEN	MIC2

Table 12: Surface Antigens of Human Chromosome 11

Long Name	Abbreviation
WILMS TUMOR 1	WT1
HEMOGLOBIN--BETA LOCUS	HBB
ATAXIA-TELANGIECTASIA	AT
LETHAL ANTIGEN-A1	AL-A1
CD59 ANTIGEN P18-20	CD59
THY-1 T-CELL ANTIGEN	THY1
CD44 ANTIGEN	CD44
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
FUCOSYLTRANSFERASE 4	FUT4
KANGAI 1	KAI1
HEMOPHILIA A	
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 2	MS4A2
LYMPHOCYTE ANTIGEN CD5	CD5
TYROSINE HYDROXYLASE	TH
HEPATOCELLULAR CARCINOMA	
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 1	MS4A1
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
RETINOBLASTOMA	RB1

CD151 ANTIGEN	CD151
ANTIGEN MSK39 IDENTIFIED BY MONOCLONAL ANTIBODY 5.1H11	MSK39
HEMOCHROMATOSIS	HFE
INTEGRIN, BETA-3	IGB3
RED BLOOD CELL ANTIGEN MER 2	MER2
TUMOR PROTEIN p53	TP53
VON WILLEBRAND DISEASE	
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
WISKOTT-ALDRICH SYNDROME	WAS
ADENOSINE DEAMINASE	ADA
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
ALZHEIMER DISEASE	AD
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, AA	HLA-A
FLOTILLIN 2	FLOT2
ANTIGEN DEFINED BY MONOCLONAL ANTIBODY F10.44.2	
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
SURFACE ANTIGEN, GLYCOPROTEIN 75	
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	

INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
T-CELL ANTIGEN RECEPTOR, DELTA SUBUNIT	TCRD
INTEGRIN, BETA-2	IGB2
FACTOR V DEFICIENCY	
CD4 ANTIGEN	CD4
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
SURFACE ANTIGEN MIC2	MIC2

Table 13: Surface Antigens of Human Chromosome 12

Long Name	Abbreviation
VON WILLEBRAND DISEASE	
CD9 ANTIGEN	CD9
CD4 ANTIGEN	CD4
SURFACE ANTIGEN OF ACTIVATED B CELLS, BB1	BB1
HEMOPHILIA A	
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
RETINOBLASTOMA	RB1
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
CD69 ANTIGEN	CD69
SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 6	STAT6
COMPLEMENT COMPONENT C1r DEFICIENCY	
TUMOR REJECTION ANTIGEN 1	TRA1

ATAXIA-TELANGIECTASIA	AT
WISKOTT-ALDRICH SYNDROME	WAS
WILMS TUMOR	WT1
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
HEMOGLOBIN--BETA LOCUS	HBB
ALZHEIMER DISEASE	AD
TUMOR PROTEIN p53	TP53
TYRO PROTEIN TYROSINE KINASE-BINDING PROTEIN	TYROBP
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
INTEGRIN, BETA-2	ITGB2
HEMOCHROMATOSIS	HFE
FLOTILLIN 2	FLOT2
INTEGRIN, BETA-3	ITGB3
INTERFERON, GAMMA, RECEPTOR 1	INFNGR1
CD44 ANTIGEN	CD44
THY-1 T-CELL ANTIGEN	THY1
CENTROMERIC PROTEIN C1	CENPC1
ADENOSINE DEAMINASE	ADA
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
BLOOD GROUP--LUTHERAN SYSTEM	LU

SURFACE ANTIGEN MIC2	MIC2
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
LETHAL ANTIGEN-- A1	AL-A1
XG BLOOD GROUP SYSTEM	XG; PBDX
ANTITHROMBIN III DEFICIENCY	
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
HYPOPHOSPHATEMIA, X-LINKED	
FACTOR V DEFICIENCY	
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
CD59 ANTIGEN P18-20	CD59
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
CD36 ANTIGEN	CD36
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
IMMUNODEFICIENCY WITH HYPER-IgM	

Table 14: Surface Antigens of Human Chromosome 13

Long Name	**Abbreviation**
RETINOBLASTOMA	RB1
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
HEMOPHILIA A	
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
HEMOGLOBIN--BETA LOCUS	HBB

BARE LYMPHOCYTE SYNDROME, TYPE 11	
HEMOCHROMATOSIS	HFE
ATAXIA-TELANGIECTASIA	AT
ALZHEIMER DISEASE	AD
WISKOTT-ALDRICH SYNDROME	WAS
TUMOR PROTEIN p53	TP53
IMMUNODEFICIENCY WITH HYPERI-IgM	
WILMS TUMOR 1	WT1
INTEGRIN, BETA-3	ITGB3
CHEDIAK-HIGASHI SYNDROME	CHS1
FACTOR V DEFICIENCY	
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
CD59 ANTIGEN P18-20	CD59
BLOOD GROUP--LUTHERAN SYSTEM	LU
INTEGRIN, BETA-2	ITGB2
BLOOD GROUP-MN LOCUS	MN
ADENOSINE DEAMINASE	ADA
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
VON WILLEBRAND DISEASE	

SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
THY-1 T-CELL ANTIGEN	THY1
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
THYMOCYTE ANTIGEN CD1A	CD1A
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
LETHAL ANTIGEN- A1	AL-A1
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
HISTOCOMPATIBILITY Y ANTIGEN	HY
SURFACE ANTIGEN MIC2	MIC2
CD44 ANTIGEN	CD44
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
ANTITHROMBIN III DEFICIENCY	
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
CD ANTIGEN	CD
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
SOLUTE CARRIER FAMILY 3, MEMBER 1	SLC3A1
INTERLEUKIN 4 RECEPTOR	IL4R

TYROSINE HYDROXYLASE	TH

Table 15: Surface Antigens of Human Chromosome 14

Long Name	Abbreviation
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
ATAXIA-TELANGIECTASIA	AT
HEMOPHILIA A	
T-CELL ANTIGEN RECEPTOR, DELTA SUBUNIT	TCRD
HEMOGLOBIN--BETA LOCUS	HBB
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
RETINOBLASTOMA	RB1
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
ALZHEIMER DISEASE	AD
CYSTIC FIBROSIS TRANSMEMBRAANE CONDUCTANCE REGULATOR	CFTR
WISKOTT-ALDRICH SYNDROME	WAS
HEMOCHROMATOSIS	HFE
BONE MARROW STROMAL CELL ANTIGEN	BST1
WILMS TUMOR 1	WT1
TUMOR PROTEIN p53	TP53
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
VON WILLEBRAND DISEASE	
FACTOR V DEFICIENCY	

INTEGRIN, BETA-2	ITGB2
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
ADENOSINE DEAMINASE	ADA
CD8 ANTIGEN, ALPHA POLYPEPTIDE	CD8A
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
INTEGRIN, BETA-3	ITGB3
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 2	MS4A2
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
INTERLEUKIN 2 RECEPTOR, ALPHA	IL2RA
LETHAL ANTIGEN--A1	AL-A1
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
CD59 ANTIGEN P18-20	CD59
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
CD44 ANTIGEN	CD44
TYROSINE HYDROXYLASE	TH
IMMUNODEFICIENCY WITH HYPER-IgM	
L1 CELL ADHESION MOLECULE	L1CAM

DIPEPTIDYLPEPTIDASE IV	DPP4
XG BLOOD GROUP SYSTEM	XG; PBDX
CD9 ANTIGEN	CD9
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
BLOOD GROUP--MN LOCUS	MN
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
CD4 ANTIGEN	CD4
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
BLOOD GROUP-KELL-CELLANO SYSTEM	KEL

Table 16: Surface Antigens of Human Chromosome 15

Long Name	Abbreviation
ALANYL AMINOPEPTIDASE	ANPEP
BETA-2-MICROGLOBULIN	B2M
HEMOGLOBIN--BETA LOCUS	HBB
HEMOPHILIA A	
RETINOBLASTOMA	RB1
ATAXIA-TELANGIECTASIA	AT
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
AGGRECAN 1	AGC1
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	

HEMOCHROMATOSIS	HFE
TUMOR PROTEIN p53	TP53
CD59 ANTIGEN P18-20	CD59
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
ALZHEIMER DISEASE	AD
WILMS TUMOR 1	WT1
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
INTERLEUKIN 15	IL15
ADENOSINE DEAMINASE	ADA
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
CD44 ANTIGEN	CD44
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
WISKOTT-ALDRICH SYNDROME	WAS
IMMUNODEFICIENCY WITH HYPER IgM	
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
L1 CELL ADHESION MOLECULE	L1CAM
VON WILLEBRAND DISEASE	
INTEGRIN, BETA-2	ITGB2

BLOOD GROUP--MN LOCUS	MN
TRANSFERRIN	TF
LETHAL ANTIGEN-- A1	ALL-A1
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
HYPOPHOSPHATEMIA, X-LINKED	
FACTOR V DEFICIENCY	
CENTROMERIC PROTEIN C1	CENPC1
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
T-CELL ANTIGEN RECEPTOR, DELTA SUBUNIT	TCRD
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
LYMPHOCYTE ANTIGEN CD5	CD5
T-LYMPHOCYTE SURFACE CD ANTIGEN	CD2
INTEGRIN, BETA-3	ITGB3
BLOOD GROUP--LUTHERAN SYSTEM	LU
ANTITHROMBIN III DEFICIENCY	
CD4 ANTIGEN	CD4
CHEDIAK-HIGASHI SYNDROME	CHS1
MEMBRANE COMPONENT, CHROMOSOME 6, POLYPEPTIDE 2	M6P2
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG

Table 17: Surface Antigens of Human Chromosome 16

Long Name	Abbreviation
CD59 ANTIGEN -18-20	CD59
HEMOPHILIA A	
HEPATOCELLULAR CARCINOMA	
CD19 ANTIGEN	CD19
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
INTEGRIN, ALPHA-L	ITGAL
INTEGRIN, ALPHA-X	ITGAX
HEMOGLOBIN-BETA LOCUS	HBB
INTERLEUKIN 4 RECEPTOR	IL4R
SIALOPHORIN	SPN
ALZHEIMER DISEASE	AD
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
BARE LYMPHOCYTE SYNDROME, TYPE II	
ATAXIA-TELANGIECTASIA	AT
INTEGRIN, ALPHA-M	ITGAM
HEMOCHROMATOSIS	HFE
INTERFERON-GAMMA-INDUCIBLE PROTEIN 16	IFI16
SOLUTE CARRIER FAMILY 7, MEMBER 5	SLC7A5
RETINOBLASTOMA	RB1
WISKOTT-ALDRICH SYNDROME	WAS

BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
TUMOR PROTEIN p53	TP53
WILMS TUMOR 1	WT1
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
ADENOSINE DEAMINASE	ADA
ANTITHROMBIN III DEFICIENCY	
FACTOR V DEFICIENCY	
VON WILLEBRAND DISEASE	
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
LEUKOCYTE ADHESION DEFICIENCY, TYPE 1	LAD
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
IMMUNODEFICIENCY WITH HYPER-IgM	
CD80 ANTIGEN	CD80
LETHAL ANTIGEN-- A1	AL-A1
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
CD86 ANTIGEN	CD86
ANTIGEN DEFINED BY MONOCLONAL ANTIBODY F.10.44.2	
INTERLEUKIN 2 RECEPTOR, ALPHA	IL2RA
CD4 ANTIGEN	CD4
INTEGRIN, BETA-3	ITGB3

THY-1 T-CELL ANTIGEN	THY1
PLASMINOGEN ACTIVATOR, TISSUE	PLAT
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
SURFACE ANTIGEN MIC2	MIC2
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
CD44 ANTIGEN	CD44
CHEDIAK-HIGASHI SYNDROME	CHS1

Table 18: Surface Antigens of Human Chromosome 17

Long Name	Abbreviation
FLOTILLIN 2	FLOT2
MEMBRANE COMPONENT, CHROMOSOME 17, SURFACE MARKER 2	M17S2
ALZHEIMER DISEASE	AD
TUMOR PROTEIN p53	TP53
INTEGRIN, BETA-3	ITGB3
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
HEMOPHILIA A	
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
T-CELL ANTIGEN CD7	CD7
HEMOGLOBIN--BETA LOCUS	HBB

HUMAN T-CELL LEUKEMIA VIRUS RECEPTOR	HTLVR
SURFACE ANTIGEN 17	SA17; S9
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
PLATELET-ENDOTHELIAL CELL ADHESION MOLECULE	PECAM1
BLOOD GROUP SYSTEM SWANN	
RETINOBLASTOMA	RB1
BLOOD GROUP SYSTEM FROESE	
HEMOCHROMATOSIS	HFE
ATAXIA-TELANGIECTASIA	AT
WILMS TUMOR 1	WT1
WISKOTT-ALDRICH SYNDROME	WAS
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
VON WILLEBRAND DISEASE	
ADENOSINE DEAMINASE	ADA
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
MAJOR HISTOCOMPATABILITY COMPLEX, CLASS I, A	HLA-A
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
CD59 ANTIGEN -18-20	CD59
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
CD4 ANTIGEN	CD4
CD8 ANTIGEN, ALPHA POLYPEPTIDE	CD8A

INTEGRIN, BETA-2	ITGB2
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
IMMUNODEFICIENCY WITH HYPER-IgM	
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
SURFACE ANTIGEN 6	S6
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
TYROSINE HYDROXYLASE	TH
HEPATOCELLULAR CARCINOMA	
SURFACE ANTIGEN MIC2	MIC2
BETA-2-MICROGLOBULIN	B2M
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
FACTOR V DEFICIENCY	
CD44 ANTIGEN	CD44
LYMPHOCYTE ANTIGEN CD5	CD5
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
TRANSFERRIN RECEPTOR	TFRC
INSULIN-LIKE GROWTH FACTOR 2 RECEPTOR	IGF2R

Table 19: Surface Antigens of Human Chromosome 18

Long Name	Abbreviation
HEMOPHILIA A	
DESMOGLEIN 3	DSG3

HEMOGLOBIN--BETA LOCUS	HBB
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
NUCLEAR FACTOR OF ACTIVATED T CELLS, CYTOPLASMIC, 1	NFATC1
TUMOR PROTEIN p53	TP53
RETINOBLASTOMA	RB1
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
ATAXIA-TELANGIECTASIA	AT
CD59 ANTIGEN P18-20	CD59
WILMS TUMOR 1	WT1
VON WILLEBRAND DISEASE	
WISKOTT-ALDRICH SYNDROME	WAS
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
ALZHEIMER DISEASE	AD
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
HEMOCHROMATOSIS	HFE
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
CHEDIAK-HIGASHI SYNDROME	CHS1
INTEGRIN, BETA-2	ITGB2
BLOOD GROUP--MN LOCUS	MN
IMMUNODEFICIENCY WITH HYPER-IgM	
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD

INTEGRIN, BETA-3	ITGB3
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
HYPOPHOSPHATEMIA, X-LINKED	
PLASMINOGEN ACTIVATOR, TISSUE	PLAT
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
XG BLOOD GROUP SYSTEM	XG; PBDX
TYROSINE HYDROXYLASE	TH
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
CD4 ANTIGEN	CD4
CD44 ANTIGEN	CD44
ADENOSINE DEAMINASE	ADA
THY-1 T-CELL ANTIGEN	THY1
FACTOR V DEFICIENCY	
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
L1 CELL ADHESION MOLECULE	L1CAM
DISACCHARIDE INTOLERANCE I	
HISTOCOMPATIBILITY Y ANTIGEN	HY
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6

RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
BARE LYMPHOCYTE SYNDROME, TYPE II	
CD36 ANTIGEN	CD36
TRANSFERRIN	TF

Table 20: Surface Antigens of Human Chromosome 19

Long Name	Abbreviation
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
BLOOD GROUP-LUTHERAN SYSTEM	LU
PREGNANCY-SPECIFIC BETA-1-GLYCOPROTEIN 2	PSG2
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
POLIO VIRUS RECEPTOR	PVR
HEMOGLOBIN--BETA LOCUS	HBB
ALZHEIMER DISEASE	AD
HEMOPHILIA A	
FUCOSYLTRANSFERASE 1	FUT1
CD79A ANTIGEN	DC79A
BARE LYMPHOCYTE SYNDROME TYPE II	
RETINOBLASTOMA	RB1
TUMOR PROTEIN p53	TP53

HEMOCHROMATOSIS	HFE
WILMS TUMOR 1	WT1
BASIGIN	BSG
KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTOR, TWO DOMAINS, SHORT CYTOPLASMIC TAIL, 1	KIR2DS1
PLASMINOGEN ACTIVATOR RECEPTOR, UROKINASE-TYPE	PLAUR
TYRO PROTEIN TYROSINE KINASE-BINDING PROTEIN	TYROBP
BLOOD GROUP-OK	OK
WISKOTT-ALDRICH SYNDROME	WAS
L1 CELL ADHESION MOLECULE	L1CAM
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
INTEGRIN, BETA-3	ITGB3
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
INTEGRIN, BETA-1	ITGB1
CD59 ANTIGEN P18-20	CD59
VON WILLEBRAND DISEASE	
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
IMMUNODEFICIENCY WITH HYPER-IgM	
CD44 ANTIGEN	CD44
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
ATAXIA-TELANGIECTASIA	AT
ANTITHROMBIN III DEFICIENCY	

MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
CHEDIAK-HIGASHI SYNDROME	CHS1
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
THY-1 T-CELL ANTIGEN	THY1
TRANSFERRIN	TF
MEMBRANE-SPANNING 4 DOMAINS, SUBFAMILY A, MEMBER 1	MS4A1
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
TRANSFERRIN RECEPTOR	TFRC
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
FUCOSYLTRANSFERASE 4	FUT4
ADENOSINE DEAMINASE	ADA

Table 21: Surface Antigens of Human Chromosome 20

Long Name	**Abbreviation**
CD59 ANTIGEN P18-20	CD59
ADENOSINE DEAMINASE	ADA
HEMOGLOBIN--BETA LOCUS	HBB
RETINOBLASTOMA	RB1
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR

MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
PROTEIN-TYROSINE PHOSPHATASE, NONRECEPTOR TYPE, SUBSTRATE 1	PTPNS1
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 5	TNFRSF5
CENTROMERIC PROTEIN B	CENPB
ATAXIA-TELANGIECTASIA	AT
HEMOPHILIA A	
HEMOCHROMATOSIS	HFE
WILMS TUMOR 1	WT1
VON WILLEBRAND DISEASE	
TUMOR PROTEIN p53	TP53
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
INTEGRIN, BETA-3	ITGB3
PHOSPHODIESTERASE I/NUCLEOTIDE PYROPHOSPHATASE 1	PDNP1
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
ALZHEIMER DISEASE	AD
FACTOR V DEFICIENCY	
WISKOTT-ALDRICH SYNDROME	WAS
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
MEMBRANE COMPONENT, CHROMOSOME 1, SURFACE MARKER 1	M1S1
CD44 ANTIGEN	CD44
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6

IMMUNODEFICIENCY WITH HYPER-IgM	
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I	HLA-A
FUCOSYLTRANSFERASE 4	FUT4
ANTITHROMBIN III DEFICIENCY	
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
INTERCELLULAR ADHESION MOLECULE 1	ICAM1
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
PLASMINOGEN ACTIVATOR, TISSUE	PLAT
BLOOD GROUP--MN LOCUS	MN
THY-1-CELL ANTIGEN	THY1
0TRANSFERRIN	TF
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
XG BLOOD GROUP SYSTEM	XG; PBDX
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG2	ERBB2
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
INTERLEUKIN 4 RECEPTOR	IL4R
INSULIN-LIKE GROWTH FACTOR 2 RECEPTOR	IGF2R
CENTROMERIC PROTEIN C1	CENPC1
DIPEPTIDYLPEPTIDASE IV	DPP4

LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE

Table 22: Surface Antigens of Human Chromosome 21

Long Name	Abbreviation
ALZHEIMER DISEASE	AD
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
INTEGRIN, BETA-2	INTGB2
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
RETINOBLASTOMA	RB1
SURFACE ANTIGEN 21	S14
HEMOPHILIA A	
HEMOGLOBIN--BETA LOCUS	HBB
ATAXIA-TELANGIECTASIA	AT
HEMOCHROMATOSIS	HFE
IMMUNODEFICIENCY WITH HYPER-IgM	
TUMOR PROTEIN p53	TP53
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
XG BLOOD GROUP SYSTEM	XG; PBDX
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS

VON WILLEBRAND DISEASE	
WILMS TUMOR 1	WT1
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2	ERBB2
THY-1 T-CELL ANTIGEN	THY1
PLASMINOGEN ACTIVATOR, TISSUE	PLAT
MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, A	HLA-A
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	HLA-A
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
EPIDERMAL GROWTH FACTOR RECEPTOR	EGFR
T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
INTEGRIN, BETA-3	ITGB3
TRANSFERRIN	TF
CD59 ANTIGEN P18-20	CD59
WISKOTT-ALDRICH SYNDROME	WAS
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
BLOOD GROUP--MN LOCUS	MN
CD9 ANTIGEN	CD9
ADENOSINE DEAMINASE	ADA
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
CHEDIAK-HIGASHI SYNDROME	CHS1
CD83 ANTIGEN	CD83

DISACCHARIDE INTOLERANCE I	
FACTOR V DEFICIENCY	
FLAUJEAC FACTOR DEFICIENCY	
TYROSINE HYDROXYLASE	TH
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
CD36 ANTIGEN	CD36
ANTITHROMBIN III DEFICIENCY	
L1 CELL ADHESION MOLECULE	L1CAM
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
PLATELET-ENDOTHELIAL CELL ADHESION MOLECULE	PECAM1
INTEGRIN, ALPHA-L	ITGAL

Table 23: Surface Antigens of Human Chromosome 22

Long Name	Abbreviation
HEMOPHILIA A	
HEMOGLOBIN--BETA LOCUS	HBB
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
INTERLEUKIN 2 RECEPTOR, BETA	IL2RB
RETINOBLASTOMA	RB1
ALZHEIMER DISEASE	AD
ATAXIA-TELANGIECTASIA	AT
WISKOTT-ALDRICH SYNDROME	WAS

HEMOCHROMATOSIS	HFE
TUMOR PROTEIN p53	TP53
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPES	
WILMS TUMOR 1	WT1
MAJOR HISTOCOMPATABILITY COMPLEX, CLASS I, A	HLA-A
BETA-2 MICROGLOBULIN	B2M
FLOTILLIN 2	FLOT2
CD59 ANTIGEN P18-20	CD59
IMMUNODEFICIENCY WITH HYPER-IgM	
VON WILLEBRAND DISEASE	
ADENOSINE DEAMINASE	ADA
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
INTEGRIN, BETA-3	ITGB3
LETHAL ANTIGEN-A1	AL-A1
INTERFERON, GAMMA, RECEPTOR 1	IFNGR1
T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT	TCRA
XG BLOOD GROUP SYSTEM	XG; PBDX
L1 CELL ADHESION MOLECULE	L1CAM
THROMBASTHENIA OF GLANZMANN AND NAEGELI	
FACTOR V DEFICIENCY	
PLASMINOGEN ACTIVATOR, TISSUE	PLAT

T-CELL ANTIGEN RECEPTOR, GAMMA SUBUNIT	TCRG
INTEGRIN, BETA-2	ITGB2
LEUKOCYTE ADHESION DEFICIENCY, TYPE I	LAD
LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1	SLC4A1
TYROSINE HYDROXYLASE	TH
HYPOPHOSPHATEMIA, X-LINKED	
BLOOD GROUP--MN LOCUS	MN
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6	TNFRSF6
BARE LYMPHOCYTE SYNDROME, TYPE II	
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
TRANSFERRIN RECEPTOR	TFRC
RHESUS BLOOD GROUP, CcEe ANTIGENS	RHCE
SURFACE ANTIGEN 21	S14
CD86 ANTIGEN	CD86
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
BLOOD GROUP--LUTHERAN SYSTEM	LU
CD36 ANTIGEN	CD36
BULLOUS PEMPHIGOID ANTIGEN 1	BPAG1
FLAUJEAC FACTOR DEFICIENCY	

Table 24: Surface Antigens of Human Chromosome X

Long Name	Abbreviation
BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE	BTK
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER TYPE	
SEVERE COMBINED IMMUNODEFICIENCY, X-LINKED	SCIDX1
IMMUNODEFICIENCY WITH HYPER-IgM	
WISKOTT-ALDRICH SYNDROME	WAS
HYPOPHOSPHATEMIA, X-LINKED	
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
HEMOPHILIA A	
XG BLOOD GROUP SYSTEM	XG; PBDX
SURFACE ANTIGEN, X-LINKED	SAX
ATAXIA-TELANGIECTASIA	AT
INTEGRIN, ALPHA-X	ITGAX
RETINOBLASTOMA	RB1
INTERLEUKIN 2 RECEPTOR, GAMMA	IL2RG
L1 CELL ADHESION MOLECULE	L1CAM
HEMOGLOBIN--BETA LOCUS	HBB
SURFACE ANTIGEN MIC2	MIC2
V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG	HRAS
TUMOR PROTEIN p53	TP53
VON WILLEBRAND DISEASE	

MIC2 SURFACE ANTIGEN, Y-CHROMOSOMAL	MIC2Y
LEUKOCYTE ADHESION DEFICIENCY, TYPE 1	LAD
WILMS TUMOR 1	WT1
INTEGRIN, BETA-2	ITGB2
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
SHORT STATURE	SS
HISTOCOMPATIBILITY Y ANTIGEN	HY
HOMEO BOX GENE HB9	HLXB9
CENTROMERIC PROTEIN C1	CENPC1
BLOOD GROUP-KELL-CELLANO SYSTEM	KEL
FUCOSYLTRANSFERASE 4	FUT4
DIPEPTIDYLPEPTIDASE IV	DPP4
BARE LYMPHOCYTE SYNDROME, TYPE II	
ADENOSINE DEAMINASE	ADA
LEUKOCYTE ADHESION DEFICIENCY, TYPE II	
SIALOPHORIN	SPN
CATHEPSIN E	CTSE
ANTITHROMBIN II DEFICIENCY	
FUCOSYLTRANSFERASE 1	FUT1
INTEGRIN, ALPHA-L	ITGAL
TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 5	TNFRSF5
SOLUTE CARRIER FAMILY 3, MEMBER 1	SLC3A1
XG REGULATOR	XGR

Fc FRAGMENT OF IgE, HIGH AFFINITY I, RECEPTOR FOR, ALPHA SUBUNIT	FCER1A

Table 25: Surface Antigens of Human Chromosome Y

Long Name	Abbreviation
HISTOCOMPATABILITY Y ANTIGEN	HY
XG BLOOD GROUP SYSTEM	XG; PBDX
MIC2 SURFACE ANTIGEN MIC2	MIC2
ATAXIA-TELANGIECTASIA	AT
HEMOPHILIA A	
SOLUTE CARRIER FAMILY 3, MEMBER 2	SLC3A2
HEMOGLOBIN--BETA LOCUS	HBB
RETINOBLASTOMA	RB1
CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	CFTR
HEMOCHROMATOSIS	HFE
IMMUNODEFICIENCY WITH HYPER-IgM	
CHEDIAK-HIGASHI SYNDROME	CHS1
RHESUS BLOOD GROUP-ASSOCIATED GLYCOPROTEIN	RHAG
DIPEPTIDYLPEPTIDASE IV	DPP4
SHORT STATURE	SS
DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED	
FACTOR V DEFICIENCY	
CD36 ANTIGEN	CD36

LUPUS ERYTHEMATOSUS, SYSTEMIC	SLE
CD24 ANTIGEN	CD24
BASIGIN	BSG

Examples of non-surface antigen proteins that may also be used as a linked gene with this method are thymidine kinase, encoded by a gene on Human Chromosome 17 and HRPT, encoded by a gene on Human Chromosome X. Selection of cells expressing these and similar linked genes could be accomplished through antibody analysis, but it might also be accomplished through simple cell culture in selective medium.

Separation of Cells Expressing Linked Genes

After genetic transfer of the test gene, it would be optimal to separate the cells which have taken up the DNA from those which have not. The mixed population may be isolated through known selection processes or through the expression of the linked gene. This ensures that the cell used for the following functional assays actually contains copy of the test gene.

If the linked gene encodes a surface antigen, target cells may be identified by incubation with fluorescently labeled antibodies. These cells may be detected through microscopy or FACS analysis. Additionally, they may be sorted from a non-expressing population of cells.

Panning and immunoprecipitation or precipitation through magnetic beads may serve as alternatives to FACS for separating cells that have received the test gene. These methods might be used as described in Small, M., *et al.*, "Isolation of CD3-, CD4-, CD8-, IL-2R+ thymocyte precursors by panning", *J. Immunol. Methods 167 (1-2)*: 103-107 (1994); Hoogenboom, H.R., *et al.*, "Selection-dominant and nonaccessible epitopes on cell-surface receptors revealed by cell-panning with a large phage antibody library", *Eur. J. Biochem. 260(3)*: 774-84 (1999); Wysocki, L.J. and Sato, V.L. "'Panning' for lymphocytes: a method for cell selection", *Proc. Natl. Acad. Sci. U.S.A. 75(6)*: 2844-2848 (1978); and Maryanski, J.L., *et al.*, "A simple panning

method for the selection of cell surface antigen transfectants", *J. Immunol. Methods 79(1)*: 159-163 (1985), incorporated by reference herein. Briefly, in the panning method a glass or plastic surface might be coated with a substance, such as an antibody, that will bind with a linked surface gene. Thus, cells expressing the linked surface gene could be separated from those that did not. After panning one could examine the target cells microscopically for the target gene. It may additionally be possible to use one linked gene for the panning analysis and a second linked gene for the microscopic assay.

For separation by immunoprecipitation or magnetic beads, the beads may be coated with a ligand, antigen or antibody so that only cells positive for a particular surface marker will be bound to the beads. Cells expressing the linked surface marker may then be isolated from other target cells through separation of the beads into new medium. The target cells could then be further examined for receipt of the test gene or expression of the test protein on the beads or after separation from the beads. Some potential techniques of this nature are described in Jurman, M.E., *et al.*, "Visual identification of individual transfected cells for electrophysiology using antibody-coated beads", *Biotechniques 17(5)*: 876-881 (1994); Thomas, T.E. *et al.*, "Specific binding and release of cells from beads using cleavable tetrameric antibody complexes", *J. Immunol. Methods 120(2)*: 221-231 (1989); Partington, K.M., ., "A novel methods of cell separation based on dual parameter immunomagnetic cell selection", *J. Immunol. Methods 223(2)*: 195-205 (1999); Patel, D. and Rickwood, D., "Optimization of conditions for specific binding of antibody-coated beads to cells", *J. Immunol. Methods 184(1)* : 71-80 (1995); Pilling, D., *et al.*, "The kinetics of interaction between lymphocytes and magnetic polymer particles", *J. Immunol. Methods 122(2)*: 235-41 (1989); Widjojoatmodjo, M.N., *et al.*, "Comparison of immunmagnetic beads coated with protein A, protein G, or goat anti-mouse immunoglobulins. Applications in enzyme immunoassays and immunomagnetic separations", *J. Immunol. Methods. 165(1)*: 11-19 (1993); and Vaccare, D.E., "Applications of magnetic separation: cell sorting", *Am. Biotechnol. Lab. 8(5)*: 32-35 (1990), incorporated by reference herein.

Fluorescently labeled antibodies may also be used to detect linked genes which are expressed intracellularly. However, such proteins may be more readily detectable by functional assays. Such assays will vary as greatly as the linked proteins. However, useful assays similar to those described below for detection of the test protein will be appropriate. Functional assays may also be useful in combination with surface antigens. Any assay, whether functional or antigen-based is appropriate so long as it detects expression of the linked gene.

Functional Assays for Test Gene Products

In a most preferred embodiment of the invention, the hybrid target cell population is evaluated for presence of the test gene by analysis of the function of the test protein. This testing may also be accomplished by a functional assay that allows functional protein to be distinguished from mutant forms that may be non-functional or partially functional if a loss-of-function mutation, including a partial loss-of-function mutation, an alteration of function mutation or a dominant negative mutation is present or additionally functional if a gain-of-function mutation is present. Functional analysis of the expressed protein may also be accomplished by an assay which detects the restoration of a function in target cells which are deficient in that function.

Assays currently in development may also be used with the method of this invention to detect cells that have received the test gene. These assays may also prove useful for detection of expression of a functional test gene product. Some such assays include those described in Bildiriel, L. and Rickwood, D., "Fractionation of differentiating cells using density perturbation", *J. Immunol. Methods 240(1-2)*: 93-99 (June, 2000); Perrin, A. *et al.*, "Immunomagnetic concentration of antigens and detection based on a scanning force microscopic immunoassay", *J. Immunol. Methods 224(1-2)*: 77-87 (1999); and Schmitz, B., *et al.*, "Magnetic activated cell sorting (MACS) - a new immunomagnetic method for megakaryocytic cell isolation: comparison of different separation techniques", *Eur. J. Immunol. 52(5)*: 267-275 (1994), incorporated herein by reference for use in detection of both cells that received the test gene and cells in which a functional test gene product is expressed.

This invention provides a more economical or efficient means of detecting heterozygous loss-of-function or gain-of-function mutations than other methods presently available. It additionally may be adapted in many ways to optimize its utility for detecting a particular heterozygous loss-of-function or gain-of-function mutation including partial loss-of-function, alteration of function and dominant negative mutations based on the function of the wild type and /or mutant proteins. The sensitivity and specificity of any particular test can be determined by how well the method of detection of the test protein mimics or parallels the function of the gene *in vivo*.

In a preferred embodiment of the invention, target cells are analyzed not only for presence of the test gene, but also for its expression and the function of the expressed protein. The optimal goal of such analysis is to detect the function of the expressed test protein in a manner as analogous to the *in vivo* situation as possible. For some test genes, it may be more appropriate to distinguish between wild type or functional mutant alleles and loss-of-function or gain-of-function mutant alleles using immunological analysis. Table 26 lists diseases related to loss-of-function or gain-of-function mutations, appropriate target cells for such disease, and appropriate assays. More detailed descriptions of some assays are provided below. Table 26 and the descriptions below are not intended to describe all assays that may be used to detect expression or function of a test protein. Many other functional or expression-based assays may be more appropriate for other test genes, as will be appreciated by one skilled in the art.

Table 26: Potential Target Diseases, Related Genes and Mutations and Assays

Disorder	Gene(s) and Known Mutations	Target Cells	Assays
Familial hypercholsterolemia	LDLR; loss-of-function	LDLR deficient CHO cells	Uptake of fluorescent LDL
HNPCC	MSH1, MSH2, PMS1, PMS2; loss-of-function	MSH1, MSH2, PMS1, PMS2 deficient mouse or human cells; mismatch repair deficient mouse, human or yeast cells	Mismatch repair functional assay
Breast or ovarian cancer	BRCA1; BRCA2; loss-of-function	BRCA1/BRCA2 deficient mouse or human cells	2-hybrid inhibition or immunological assay
Neurofibromatosis	NF1; NF2; loss-of-function	NF1, NF2 deficient mouse or human cells	2-hybrid inhibition or immunological assay
Polyposis of the colon	APC; loss-of-function	APC deficient mouse or human cells	2-hybrid inhibition or immunological assay

Duchenne dystrophy	Dystrophin genes; loss-of-function	Dystrophin deficient myoblasts	immunodetection of dystrophin complex or functional assay; *in vivo* assay
Cystic fibrosis	CFTR; loss-of-function	CFTR deficient cells; CFPAC-1	Ion channel activity assay
Li Fraumenti	loss-of-function; possible gain-of-function		2-hybrid inhibition or immunological assay
Tuberous sclerosis	loss-of-function		2-hybrid inhibition or immunological assay
Gorlin syndrome	loss-of-function		2-hybrid inhibition or immunological assay
Von Hippel-Lindau	loss-of-function		2-hybrid inhibition or immunological assay

Porphyrias	loss-of-function		Histochemistry or 2-hybrid inhibition or immunological assay
Osteogenesis imperfecta	loss-of-function; gain-of-function		2-hybrid inhibition or immunological assay
Marfan	possible loss-of-function; -gain of-function		2-hybrid inhibition or immunological assay
Hemophilia	loss-of-function		Coagulant activity of 2-hybrid inhibition or immunological assay
SCID	loss-of-function		2-hybrid inhibition or immunological assay

Functional Assay: Endocytic Uptake of Ligand

For test genes encoding a receptor, functional analysis may comprise an assay to detect normal interaction of that receptor with its ligand. If a fluorescently labeled ligand is used, cells may then be examined for its binding or uptake via microscopy or FACS. For example, to detect a defect in the LDLR (low density lipoprotein receptor), target cells containing the test gene may be incubated with

commercially available, fluorescently labeled LDL (low density lipoprotein). (See Corsetti, J.P. *et al.*, "The labeling of lipoproteins for studies of cellular binding with a fluorescent lipophilic dye", *Anal. Biochem. 195*: 122 (1991), incorporated by reference herein, for a description of this technique.) Target cells in which the test protein is expressed and functions normally will internalize the labeled LDL while those with loss-of-function mutations will not. (This is demonstrated in the Examples below.) Cells that have internalized the LDL are visible through microscopy. For the protein to carry out this function, it must be synthesized, stable, properly processed and capable of ligand binding and it must be able to carry out the normal internalization function. Rare mutations that permit ligand binding but block internalization might also be detectable by this technique, as cells expressing such mutant alleles will show LDL staining at the plasma membrane, but not in the cytoplasm.

One surface antigen, ICAM-1 (intercellular adhesion molecule-1) is known to be linked to test gene, LDLR. In a more preferred embodiment, microcell-mediated chromosome transfer (MMCT) is used to transfer Human Chromosome 19 from lymphoblast donor cells to Chinese Hamster Ovary (CHO) target cells. Chromosome 19 comprises the test gene which encodes human LDLR and the gene that encodes ICAM-1. The ICAM-1 gene is naturally linked to the LDLR gene such that separation by recombination or chromosome damage is unlikely. After genetic transfer, target cells are incubated with fluorescently labeled LDL under conditions that allow LDL binding to wild type or functional LDLR and endocytic uptake by the cells. Cells in which the LDLR gene is functional exhibited cytoplasmic staining whereas those with a LDLR loss-of-function mutation are not labeled. Cells with an LDLR partial loss-of-function mutation may exhibit reduced cytoplasmic staining, only surface staining or no staining. In addition, target cells can be labeled with an anti-ICAM-1 antibody. Positively stained ICAM cells may be sorted from unlabelled cells. Slides of the target cells are then prepared and fluorescent microscopy used to visualize cells labeled with the ICAM-1 antibody and those labeled by uptake of fluorescent LDL such that the ratio of cells that express functional LDLR to the total number of cells that received the test gene and express ICAM-1 may be calculated.

LDLR linkage to ICAM-1 allows the application of both immunodetection of linked surface antigen and a functional assay of the test gene (endocytic LDL uptake).

Functional Assay:Ion Channel Activity

The CFTR (Cystic Fibrosis Transmembrane Conductance Regulator Protein) gene is another potential test gene in this method. Loss-of-function mutations in the CFTR gene can lead to cystic fibrosis in individuals homozygous for such mutations. Early and efficient detection of these individuals can lead to more effective treatment of the disease. Additionally, detection of individuals heterozygous for a CFTR loss-of-function mutation is useful for medical and research purposes and especially for genetic counseling. Since CFTR encodes a chloride channel, a relevant functional assay must measure ion transport through electrophysiological techniques. One such functional assay is described in Mansoura, M.K. *et al.*, "Fluorescent chloride indicators to assess the efficacy of CFTR cDNA delivery", *Hum. Gene Ther. 10(6)*: 861-75 (1999), incorporated herein by reference.

Functional Assay: Mismatch Repair

HNPCC (hereditary nonpolyposis colon cancer) is caused by mutations in DNA mismatch repair genes, e.g. MLH1, MSH1, PMS1, PMS2. Any of these mutations may be detected through the use of a mismatch repair functional assay. The chosen target cells must be mismatch repair deficient. If a test gene, associated with HNPCC, encodes for a functional mismatch repair protein, its expression in the target cells should restore mismatch repair. Cells, which incorporated a mutant allele of an HNPCC associated gene, will continue to be defective in mismatch repair. Restoration of function may be detected through the use of various reporter gene system known in the art. Figure 4 depicts one potential mismatch repair assay. A variety of other tests which analyze for mismatch repair are described in Corrette-Bennet, S.E. and Lahue, R.S., "Mismatch Repair Assay", *Methods Mol. Biol. 113*: 121 (1999); Bill, C.A. *et al.*, "Efficient repair of all types of single-base mismatched in recombination intermediates in Chinese hamster ovary cells. Competition between long-patch and G-T glycosylase-mediated repair of G-T mismatches", *Genetics 149*: 1935 (1998); Varlet, I., *et al.*, "DNA mismatch repair in

Xenopus egg extracts; repair efficiency and DNA repair synthesis for all single base-pair mismatches", *Proc. Natl. Acad. Sci. U.S.A. 87*: 7883 (1990); and Shimodaira, H. *et al.*, *Nat. Genet. 19*: 384 (1998), incorporated herein by reference. Yeast cells might prove particularly attractive for this type of testing. An exemplary mismatch repair assay in yeast cells is described in Shimodaira, H., *et al.*, "Functional Analysis of Human MLH1 mutations in *Saccharomyces cervisiae*", *Nat. Genet. 19*: 384 (1998), published erratum in *Nat. Genet. 21(2)*: 241 (1999), incorporated herein by reference. As for other genes, functional analysis assays might incorporate a variety of detection formats including microscopy, FACS, or perhaps inspection for yeast colonies on culture plates.

Functional Assay: Two Hybrid System

Since many genes associated with inherited genetic disorders have undergone extensive biochemical analysis, they have known binding partners. Thus, functional assays incorporating two hybrid analysis may be applied to practice the invention. The target cell can be analyzed for the presence of a functional copy of the test gene, if the test protein demonstrates a successful protein-protein interaction with a known binding partner. For a description of a 2-hybrid system useful in the present invention see, *e.g.* Bartel, P.L. and Fields, S., "Analyzing protein-protein interactions using a two-hybrid system", *Methods Enzymol. 254*: 241 (1995); Schwartz, H. *et al.*, "Mutation detection by a two-hybrid assay", *Hum. Mol. Genet. 7*: 1029 (1998; and Germino, F.J. and Moskowitz, N.K., "Screening for protein-protein interactions", *Methods Enzymol. 303*: 422-50 (1999), incorporated herein by reference. The use of a standard or inhibition two-hybrid assay must be designed to comprise a test system and a reporter system. The reporter system must not interfere with the test system and allow for assay of either positive or negative interactions.

More specifically, an appropriate two-hybrid assay might be developed by expressing in the hybrid target cells a fragment of the test gene fused to a DNA binding domain under the control of a constitutive promoter. The fragment of the test gene should encode at least the interactive portion the test protein for which capacity to interact with another protein is to be assayed. The target cells would also be provided with another construct that expresses at least the interactive portion of the

protein with which the test protein is to interact fused with a DNA activation domain perhaps under the control of a tetracycline-regulated promoter. These two constructs comprise the test system. The target cells should be further provided with a reporter system such that a detectable product such as GFP, luciferase, or secreted a-fetoprotein is produced only if the test protein interacts with the reporter gene product. Further, for more sensitive interference-competition assays, the fragment of the gene of interest could be chosen so as to have a somewhat weaker interaction than the full length, functional or wild type protein. In addition, the ratio of the test protein to the interacting protein could be controlled through the tetracycline promoter. Figure 5 describes a 2-hybrid assay of this type that may be used with the present invention.

While establishment of a target cell line with the appropriate test and reporter systems might require a moderate amount of time, the techniques should be routine to one skilled in the art for most potential target cells and assays. Further, the single target cell line would be useful for all individuals regarding analysis of the test gene and possibly the target disease. After establishment of the target cell line, the test gene might be delivered to is by any of the methods described above. Function of the test gene may be measured by its ability to compete with the DNA binding/protein fragment of the test system and thereby decrease expression of the reporter gene. For a test protein to compete in this type of assay, it would have to be synthesized, stable, and capable of interaction with its physiological partner, the interacting protein (reporter gene product).

Functional Assay: GFP Fusion Protein

Another assay might test for the ability of the test protein to interact with a known fluorescently tagged binding partner. If the interacting protein were expressed in the target cell as a GFP fusion protein and this binding interaction resulted in a known subcellular translocation, one would be able to detect any known changes in subcellular localization that result from a protein-protein interaction. This strategy relies on the targeting of proteins to specific subcellular locations upon a protein binding, e.g. cytoplasm to nucleus, cytoplasm to plasma membrane, nucleus to cytoplasm, etc. This assay might be developed by expanding upon the description in Sakai, N. *et al.*, "Direct visualization of the translocation of the gamma-subspecies of

protein kinase C in living cells using fusion protein with green fluorescent protein", *J. Cell. Biol. 139*: 1465 (1997), incorporated herein by reference. While Sakai *et al.* used cloned genetic material, their methods should be adaptable for use with chromosomes or large genomic DNA fragments without undue experimentation.

Functional Assay: *In Vivo*

Functional assays need not always be carried out *in vitro*. An assay to detect Duchenne dystrophy, ornithine transcarbamylase deficiency, or other disease might be performed *in vivo*. For such an assay, the target cells would be located inside a living organism. These target cells might be naturally deficient cells or cells rendered deficient through knockout techniques. The living organism might then be, for instance, a deficient strain of mice or a knockout mouse. Preparation of a knockout mouse for a test gene of interest may be accomplished through techniques currently employed in the art. In the case of Duchenne dystrophy the knockout mice would lack a dystrophin gene and the target cell would likely be a myoblast or hepatocyte. The transfer of the test gene to the target cells might be accomplished by injecting microcells prepared as in MMCT into an organ or tissue of the mouse such that the target cells would likely contact the microcells. After a few, approximately 2-5, days, the organ or tissue containing the target cell might be removed and immunohistochemistry employed to detect the functional expression product of the test gene and also a linked antigen from the same chromosome as the test gene. See Figure 6 for a description of an embodiment of this assay.

A variety of other functional assays may also be developed around the properties of individual genes using techniques known in the art. These might include assays for the ability to carry out an enzymatic activity, or assays for the ability to be modified (*e.g.* phosphorylated) by another protein.

Functional Assays: Gain of Function Mutants

Though the functional assays described above focus on detection of loss-of-function mutations, adaptations appropriate to detect gain-of-function mutations will be understood to one skilled in the art. For example, one such assay might involve the use of target cells deficient in a particular function that may be

conferred by a gain-of-function mutation. Thus, target cells in which this function is observed must have received a gain-of-function allele of the test gene while target cells in which the function is not observed must have received a wild type or functional allele of the test gene. (See Figure 7 for a gain-of-function example.) In general, assays for gain-of-function alleles may be very similar to those for loss-of-function alleles except that expression of a protein with a particular function will indicate the presence of a mutated, rather than a wild type or functional allele of the test gene. Figure 10 presents a general description of several of the embodiments of the present invention described above.

Heterozygous vs. Homozygous

In an embodiment of the invention, the genotype of the individual may be determined by evaluating the ratio of the number of cells expressing the wild type gene product to the number of cells expressing the test gene product. Though a variety of methods may be used to introduce a test gene into a target cell and to detect the presence of the test gene and its expression or protein function, ultimately two measurements must be made and compared to determine the genotype of the donor cell. First, a number of target cells that received the test gene must be determined. Second, a number of these target cells that also express wild type or functional protein must be determined.

If the ratio of the number of cells expressing functional or wild type protein to the number of cells that received the test gene is approximately 1:1, the donor cells are deemed to be homozygous wild type or without a deleterious mutation for the test gene. If the ratio of the number of cells expressing functional wild type protein to the number of cells that received the test gene and/or syntenic DNA is approximately 1:2, the donor cells are deemed to be heterozygous for a loss-of-function or gain-of-function mutation. If the ratio of the number of cells expressing functional or wild type protein to the number of cells that received the test gene is approximately 0:1, the donor cells are deemed to be homozygous for a loss-of-function or gain-of-function mutation.

Additionally, though only a single test gene is referred to throughout the specification and claims, it will be understood to one skilled in the art that more

than one test gene may be transferred to from the donor cell to the target cell. (See Figure 9 for an example of a multi-gene embodiment of this invention.) This may preferably be accomplished through simultaneous transfer of the multiple test genes. The target cells should be naturally or artificially capable of expressing all test genes and all test proteins should be detectable and distinguishable in the target cells. The target cells may be assayed for the presence of the each test gene separately, or, if the test genes are linked, one assay may confirm the presence of all test genes. Finally, different functional or immunological assays may be performed to detect functional or wild type expression for each test gene separately or, if the genes function in concert, a single assay that requires functional or wild type expression of each gene may be employed. Such an assay might prove valuable where one needs only to determine whether a loss-of-function or gain-of-function mutation exists in one of a set of genes, rather than in a single, specific gene.

Many of the assays described above are automatable for more rapid and efficient testing. For instance, haploid target cells may be subjected to all of the steps of the assay for the test gene and of the assay for the protein except the final visualization or counting step. Automated panning, immunoprecipitation or magnetic bead steps might be used to separate those cells expressing a linked surface antigen. The counting step might then be performed on such target cells placed on a microscopic slide via an automated counting system. This system might be similar to those currently used in hospitals and labs for blood counts. It might alternatively be performed by an automated FACS system. In order to obtain results as quickly as possible using an automated system or any other method of detection or calculation, a method using lymphoblasts as donor cells and MMCT as the transfer method might be optimal, as such a method should only require around nine days before obtaining results.

Additionally, the method described above it all of its permutations may be adapted for use in medical or veterinary testing for any disease mentioned herein, or any other disease resulting from or related to a loss-of-function mutation or a gain-of-function mutation. Such medical or veterinary testing may be conducted, *inter alia,* in diagnostic or professional laboratories by technicians, in hospital

laboratories, or in medical or veterinary offices. Steps of the method of this invention may be selected so as to be amendable to the test location. For instance, steps that require less precise conditions and procedures or that take less time might be most appropriate for use in medical or veterinary offices, while more rigorous conditions and procedures can often be performed in a diagnostic laboratory. Additionally, steps may be selected so as to maximize the clinical value of the information received while minimizing the cost of testing. Steps may also be selected to provide the most comprehensive amount of information about a mutation or potential mutation regardless of cost.

The method of the present invention might also be embodied in kits. These kits may be designed for research, medical, veterinary or other uses. The precise steps of the above methods may be selected so that the reagents are amenable to commercial production for a kit, so that the reagents are stable enough to be shipped and maintain a reasonable shelf-life, or so that the kit is easy to use. Other considerations specific to the test gene and the proposed use of the kit may influence the choice of method steps.

Although only preferred embodiments of the invention are specifically described above, it will be appreciated that modifications and variations of the invention are possible without departing from the spirit and intended scope of the invention.

The following non-limiting examples are provided to more clearly illustrate the aspects of the invention and are not intended to limit the scope of the invention.

EXAMPLES

Example 1: Selection of a Test Gene

Familial hypercholesterolemia results from a heterozygous or homozygous loss-of-function mutation in the LDLR gene. Approximately 1 in 500 individuals in the general population are heterozygous for the loss-of-function mutation. Molecular confirmation of the diagnosis is not readily available. This presents a problem particularly in regards to the certainty of diagnosis and counseling

of relatives of the disease sufferers. Thus, application of the methods of the invention to this disease may result in more accurate detection and better counseling of those predisposed to or with familial hypercholesterolemia. It may also result in more efficient and cost-effective diagnosis. Finally, application of the methods of this invention to study familial hypercholesterolemia in the laboratory may lead to a better understanding of the disease or more effective or specific treatments. Thus, the LDLR gene is an excellent test gene for the method of this invention.

Example 2: Selection of Donor Cells

As described above, biopsies and the products of other invasive methods have been previously used in MMCT and other cell fusion techniques to produce haploid hybrid cells. Obtaining such samples is costly, time-consuming and sometimes uncomfortable for the patient. To avoid such problems, lymphoblasts were selected as the donor cells for these experiments. Lymphoblasts are readily obtainable from whole blood, the collection of which is routine and minimally invasive. Use of whole blood also save times and money, since a portion of the sample may be used for other medical testing. Lymphoblasts may be obtained from whole blood by a variety of methods, including centrifugation in a Ficoll gradient. Finally, lymphoblasts are a useful donor cell for the LDLR gene because they constitutively express the gene. Thus, there is no necessity to reactivate a non-active gene before it may be expressed in the target cells.

Example 3: Selection of Target Cells and Test Gene Transfer Method

CHO cells were selected as the target cells because they are a hardy, readily available, and well-characterized cell line. Additionally, CHO cells do not express LDLR, but have been shown to be capable of expressing the fully functional protein in Corsetti *et al.* (1991).

MMCT was selected as the test gene transfer method, since it represents the most efficient means to date of transferring a chromosome or chromosome fragment from one cell to another. Transfer of an entire chromosome was desirable to preserve linkage of the LDLR gene to the gene for the surface antigen ICAM-1.

MMCT largely as described in Killary *et al.*, and partially as further described in Example 4 below was used to transfer human Chromosome 19 comprising the wild type LDLR gene to CHO cells. The CHO cells were then incubated with dil-LDL as in Corsetti *et al.*. After incubation, CHO cells with Chromosome 19 showed intense, cytoplasmic staining on microscope slides while a control group that did not receive Chromosome 19 showed no staining. Fluorescence was also determined by FACS (Figure 10). Finally, a mixture of 80% Chromosome 19 negative CHO cells and 20% Chromosome 19 positive CHO cells was subjected to FACS analysis. As expected, approximately 20% of the cells were fluorescent while 80% were not (Figure 11). The results confirm that CHO cells can express the test gene and perform the functional assay.

Example 4: Microcell Mediated Chromosome Transfer of Chromosome 19

Human Chromosome 19 was transferred from lymphoblasts to CHO cells using microcell mediated transfer techniques generally as described in Killary *et. al.* (1995). However, some variations of this method were used. The incorporation by reference of the Killary . paper and the descriptions here are not intended to limit the invention to the specific embodiments described. Other possible variations of MMCT or other potential transfer techniques will be understood by one skilled in the art and a nonexhaustive list is provided above.

Briefly, lymphocytes were isolated from whole blood using a Ficoll hypaque gradient. Lymphoblasts were prepared by stimulating the lymphocytes with phytohemagglutinin, followed by treatment with 1 μg/ml Colcemid for 48 hours to achieve prolonged metaphase arrest and induce micronucleation. Under these conditions, 36-56% of donor cells contained micronuceli. Donor cells were then plated onto thin plastic sheets, rounded at one end to fit the bottoms of 50 ml centrifuge tubes (termed "bullets"). Bullets were pretreated with Concanavalin A as described in Killary and Fournier (Methods 8:234-246, 1995) to adhere micronucleate populations in suspension onto plastic bullets. The plastic bullets with the cells adherent to the side were then placed vertically in a centrifuge tube (two bullets/tube) in medium containing cytochalasin B (5 ug/ml). Centrifugation in the presence of cytochalasin B results in the enucleation of micronucleate populations and

resultant enucleate whole cells (karyoplasts) and microcells pellet at the bottom of the centrifuge tube. The resulting pellets were then filtered through 5 micron and 3 micron nucleopore filters according to the method of McNeill and Brown (PNAS 77:5394-5398 (1980)) to select for microcells containing single human chromosomes and to remove whole cells that failed to enucleate and karyoplasts that contaminate the preparation. Under the conditions of MMCT, most target cells will not take up any chromosome. Only about 1 in 1000 to 1 in 10,000 target cells will take up a copy of Chromosome 19. (See results of Example 6). Very rarely, a target cell will take up more than one copy of Chromosome 19, but such events are so infrequent as to be irrelevant for the purposes of these experiments. After filtration, cells containing approximately one chromosome or chromosome fragment were retained. Figure 12 depicts MMCT as used in this example.

MMCT was performed for several peripheral blood lymphocyte samples, some from normal individuals, some from individuals known to be heterozygous for a loss-of-function mutation in LDLR, and some from individuals known to be homozygous for a loss-of-function mutation in LDLR. These samples representing three genotypes for the LDLR were then used in all of the examples described below.

Example 5: Incubation of Selected Target Cells with Labeled LDL

After MMCT, the selected CHO target cells were cultured for 48 hours then allowed to take up fluorescently labeled dil-LDL.

Example 6: Detection of the Test Gene Through Labeled ICAM-1

The target CHO cells were next assayed for surface expression of ICAM-1. ICAM-1 has been shown to be linked to LDLR. Target cells expressing ICAM-1 were assumed to have received the LDLR gene and to be capable of normal protein expression.

ICAM-1 expression was detected by incubating the cells with a FITC-labeled anti-ICAM-1 antibody. Cells positive for ICAM-1 were visualized using fluorescent microscopy. Cells positive for ICAM-1 were then sorted from the

remaining target cells by FACS. Approximately 250,000 - 2,300,000 cells were FACS-sorted. Of these only 0.08-0.7% were ICAM-1 positive.

The low percentage of ICAM-1 positive cells is likely due to the low efficiency of Chromosome 19 uptake than to any problems with cell sorting. Preliminary tests were performed to determine whether FITC-labeled ICAM-1 expressing cells were detectable and thus sortable through FACS analysis. Mouse L-cells transfected with a construct expressing ICAM-1 and nontransfected cells were incubated with FITC-labeled anti-ICAM-1 antibody. After incubation, cells not expressing ICAM-1 were easily distinguished from cells expressing the molecule by FACS analysis. (See Figures 13(a) and 13(b).) Thus, FACS is an efficient method for sorting of ICAM-1 expressing cells from non-ICAM expressing cells.

Example 7: Detection of Cytoplasmic Labeled LDL and ICAM-1 Expression

Spontaneous loss of chromosomal material can occur in CHO cells which originally expressed human chromosome 19. LDL Uptake and ICAM-1 expression was also performed using FACS analysis on the cells (Figure 14). 54% of the cells were negative for both LDL uptake and ICAM-1 expression while 26.2% of the cells labeled for both. Only a very small proportion of the cells, 65 and 13% were, express either ICAM-1 or exhibit LDL uptake.

LDL uptake and ICAM-1 expression was performed on CHO cells expressing LDLR genes from a normal donor as described above (Figure 15(a) and 15 b)). The majority of the cell population was positive for both ICAM-1 and LDL. For individuals heterozygous for a loss-of-function mutation in the LDLR gene, a portion of the target cell population was positive for both ICAM-1 and LDL, but another portion was positive only for ICAM-1 (Figure 16(a) and 16(b)). For samples from an individual homozygous for a loss-of-function mutation in the LDLR, most of the target cell population was positive only for ICAM-1 (Figure 17(a) and 17(b)). The FACS profile of the homozygous individual were similar to that of the negative control cells (Figure 18(a) and 18(b)).

Example 8: Interpretation of the Ratio of LDL positive cells to ICAM-1 positive cells

In illustrative slides prepared and visualized as described in Example 7, for CHO cells that received the LDLR gene from normal donor, every single ICAM-1 positive cell was also positive for the LDLR. Thus when the ratio of cells expressing the wild type or functional test gene to the total number of target cells that received the test gene is approximately 1:1, the donor cells may be deemed to be normal or without a deleterious mutation for the test gene.

For CHO cells that received the LDLR gene from a heterozygous loss-of-function donor and positively expressing ICAM-1, a mixture of LDLR positive and LDLR negative cells were detected. The ratio of cells expressing the LDLR to the number expressing ICAM-1 was roughly 1:2. Thus when the ratio of cells expressing the wild type or functional test gene to the total number of target cells that received the test gene is 1:2, the donor cells may be deemed to be heterozygous for the loss-of-function mutation.

Finally, for CHO cells that received the LDLR gene from a homozygous loss-of-function donor and positively expressing ICAM-1, not a single LDLR positive cell was observed. Thus when the ratio of cells expressing the wild type or functional test gene to the total number of target cells that received the test gene is approximately 0:2, the donor cells may be deemed to be homozygous for the loss-of-function mutation.

CLAIMS

1. A method of detecting a loss-of-function or gain-of-function mutation in a test gene of interest in an individual who may be heterozygous or homozygous for a genetic abnormality related to the test gene comprising:

obtaining a sample of genetic material from the individual, said sample containing the test gene of interest;

separating the genetic material into haploid sets, so as to contain single copies of the test gene of interest;

transferring the single copies of the test gene of interest to a population of target cells, which provide for expression therein of the gene resulting in a specifically detectable gene product; and

monitoring the target cells to determine the presence of a test gene or whether a functional, non-functional or additionally functional test gene product has been expressed in the target cells,

wherein the presence of a non-functional gene product in cells of the population of target cells indicates the presence of a loss-of-function mutation in the test gene of interest and

wherein the presence of an additionally functional gene product in cells of the population of target cells indicates the presence of a gain-of-function mutation.

2. The method of Claim 1, wherein the mutation of the test gene is associated with a disorder in the individual.

3. The method of Claim 1, wherein the mutation of the test gene is associated with a disorder in offspring of the individual.

4. The method of Claim 1, wherein the mutation of the test gene can cause a disorder selected from the group consisting of breast and ovarian cancer, familial hypercholesterolemia, hereditary nonpolyposis colon cancer (HNPCC), neurofibromatosis, polyposis of the colon, Duchenne dystrophy, cystic fibrosis, Li Fraumeni disease, tuberous sclerosis, Gorlin syndrome, Von Hippel-Lindau disease, porphyrias, osteogenesis imperfecta, Marfan's disease, polycystic kidney disease,

hemophilia, SCID, Rett syndrome, lysosomal diseases, and ornithine transcarbamylase (OTC) deficiency.

5. The method of claim 4, wherein the test gene is the low density lipoprotein receptor gene.

6. The method of Claim 1, wherein the sample of genetic material containing the test gene is obtained from donor cells.

7. The method of Claim 6, wherein the donor cells are human.

8. The method of Claim 6, wherein the donor cells are lymphoblasts.

9. The method of Claim 1, wherein the separation of the genetic material into haploid sets is performed within the donor cell.

10. The method of Claim 9, wherein the separation of the genetic material into haploid sets is a result of a transfer method.

11. The method of Claim 1, wherein the separation of the genetic material into haploid sets occurs after removal of the genetic material from the donor cells.

12. The method of Claim 1, wherein the target cells are selected from the group consisting of mammalian cells, insect cells and yeast cells.

13. The method of Claim 12, wherein the target cells are Chinese Hamster Ovary cells.

14. The method of Claim 1, wherein the single copies of the test gene of interest are located on a chromosome or chromosome fragment.

15. The method of Claim 5, wherein the low density lipoprotein receptor gene is located on Chromosome 19.

16. The method of Claim 1, wherein the single copies of the test gene of interest are transferred to the target cells through microcell mediated chromosome transfer.

17. The method of Claim 1, wherein the single copies of the test gene of interest are transferred to the target cells through electroporation.

18. The method of Claim 1, wherein the single copies of the test gene of interest are transferred to the target cells through liposome-mediated transfer.

19. The method of Claim 1, wherein the single copies of the test gene of interest are transferred to the target cells through somatic cell fusion.

20. The method of Claim 1, wherein the single copies of the test gene of interest are transferred to the target cells through fusion of sperm cells with the target cells.

21. The method of Claim 1, wherein the target cells naturally provide for the expression therein of the test gene.

22. The method of Claim 1, wherein the test gene product is a test protein.

23. The method of Claim 1, wherein the target cells are artificially manipulated to provide for expression therein of the test gene.

24. The method of Claim 1, wherein the target cells have been rendered incapable of expressing the ortholog of the test gene or other functionally interfering protein so as to allow detection of the detectable test gene product.

25. The method of Claim 1, wherein the target cells lack the function or functions provided by expression of a functional form of test gene product.

26. The method of Claim 1, wherein the target cells are monitored to determine whether a single copy of the test gene of interest was successfully transferred to the cells.

27. The method of Claim 26, wherein the target cells are monitored using an assay.

28. The method of Claim 27, wherein the assay is fluorescence *in situ* hybridization.

29. The method of Claim 27, wherein the assay is chromosome paint.

30. The method of Claim 27, wherein the assay detects the presence of a gene linked to the test gene in such a way that separation of the gene from the test gene is unlikely.

31. The method of Claim 27, wherein the assay is an assay for the expression product of the linked gene.

32. The methods of Claim 30 and 31, wherein the linked gene and the test gene are naturally linked on a chromosome.

33. The method of Claim 31, wherein the expression product of the linked gene is a protein.

34. The method of Claim 33, wherein the protein is a surface protein.

35. The method of Claim 34, wherein the assay comprises detection of the surface protein by a fluorescently tagged antibody to an antigen of the surface protein.

36. The method of Claim 35, wherein the surface protein is ICAM-1.

37. The method of Claim 26, wherein the target cells to which a single copy of the test gene of interest was successfully transferred are separated from target cells to which a single copy of the test gene of interest was not successfully transferred by fluorescence activated cell sorting.

38. The method of Claim 1, wherein monitoring the target cells to determine whether a functional, non-functional, or additionally functional gene product has been expressed is accomplished through a functional assay for the gene product.

39. The method of Claim 38, wherein the gene product is a receptor.

40. The method of Claim 39, wherein the functional assay comprises:

preparing a fluorescently labeled ligand capable of interaction with the receptor if the receptor is functional;

exposing the target cells to the labeled ligand in such a manner as to allow interaction of the ligand with the receptor if the receptor is functional;

detecting cells in which the ligand has interacted with the receptor.

41. The method of Claim 40, wherein microscopy is used to detect cells in which the ligand has interacted with the receptor.

42. The method of Claim 38, wherein the functional assay comprises a test for restoration of function in functionally deficient target cells.

43. The method of Claim 38, wherein the functional assay comprises a two-hybrid assay or a two-hybrid inhibition assay.

44. The method of Claim 1, wherein monitoring the target cells to determine whether a functional, non-functional, or additionally functional gene product has been expressed is accomplished through an immunological assay for the gene product.

45. The method of Claim 44, wherein the immunological assay comprises detection of the gene product with an antibody specific for the test protein.

46. The method of Claim 1, wherein the determination of whether a functional, non-functional, or additionally functional gene product has been expressed is used to further determine whether the individual is normal or heterozygous for a genetic abnormality or homozygous for a genetic abnormality.

47. The method of Claim 46, wherein expression of only a functional gene product is deemed to indicate that the individual is normal or without a mutation for the test gene.

48. The method of Claim 46, wherein expression of a functional gene product and a non-functional gene product or an additionally functional gene product is deemed to indicate that the individual is heterozygous for a genetic abnormality.

49. The method of Claim 46, wherein expression of only a non-functional gene product or only an additionally functional gene product is deemed to indicate that the individual is homozygous for a genetic abnormality.

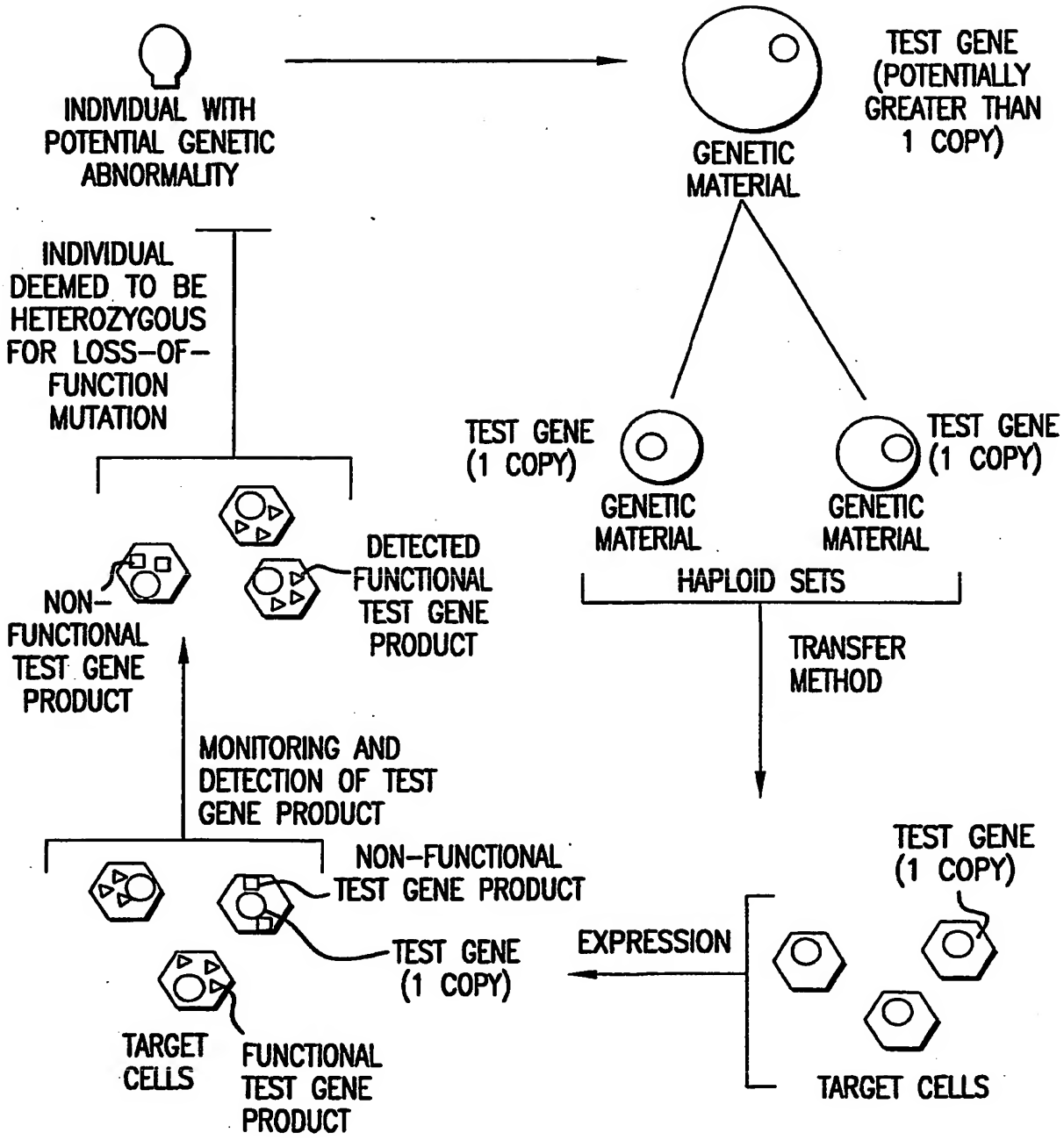
INDIVIDUAL WITH POTENTIAL GENETIC ABNORMALITY
GENETIC MATERIAL
TEST GENE (POTENTIALLY GREATER THAN 1 COPY)
INDIVIDUAL DEEMED TO BE HETEROZYGOUS FOR LOSS-OF-FUNCTION MUTATION
TEST GENE (1 COPY)
GENETIC MATERIAL
GENETIC MATERIAL
TEST GENE (1 COPY)
HAPLOID SETS
DETECTED FUNCTIONAL TEST GENE PRODUCT
NON-FUNCTIONAL TEST GENE PRODUCT
TRANSFER METHOD
MONITORING AND DETECTION OF TEST GENE PRODUCT
NON-FUNCTIONAL TEST GENE PRODUCT
TEST GENE (1 COPY)
EXPRESSION
TEST GENE (1 COPY)
TARGET CELLS
FUNCTIONAL TEST GENE PRODUCT
TARGET CELLS

FIG.1

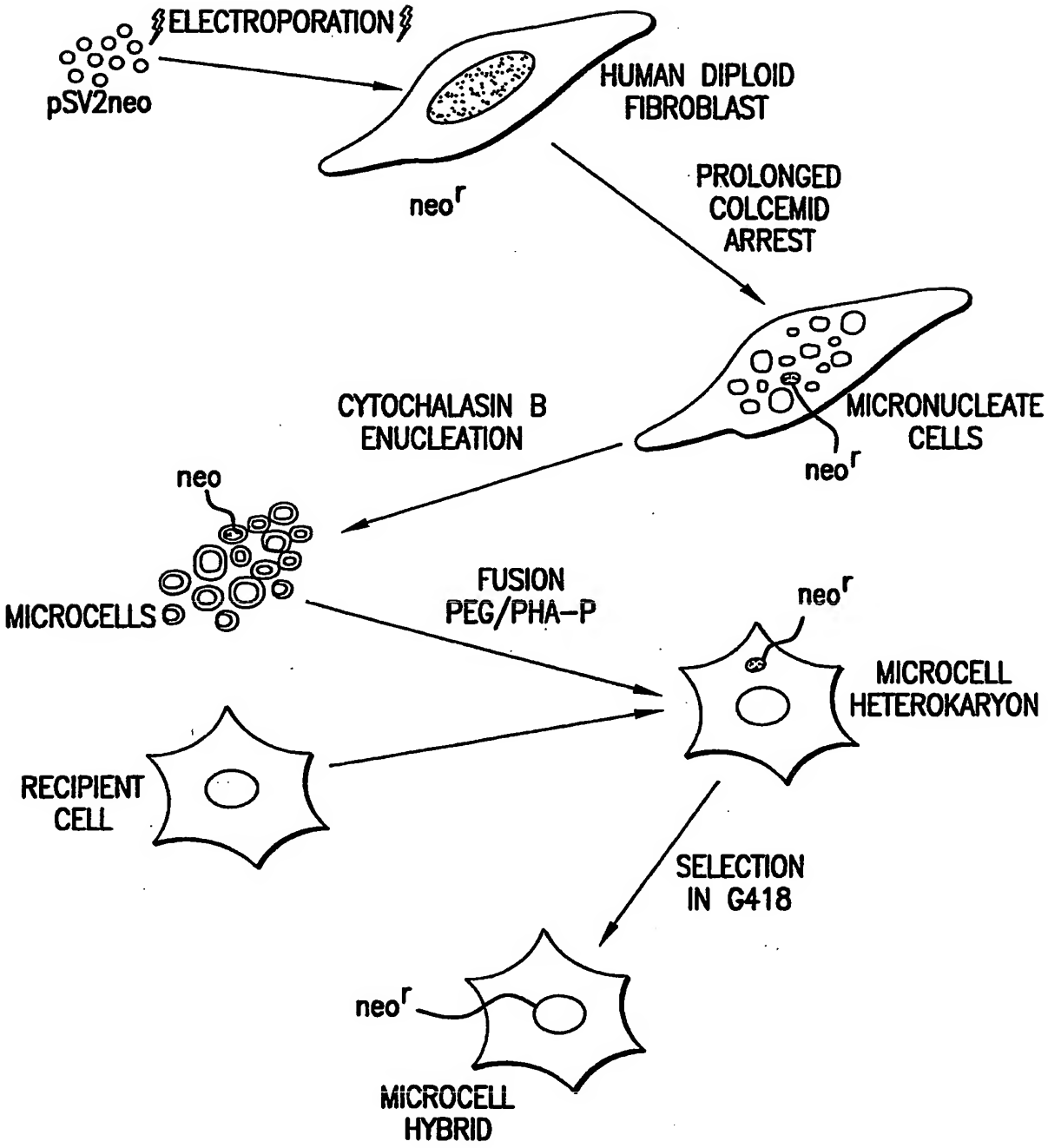
ELECTROPORATION
pSV2neo
HUMAN DIPLOID FIBROBLAST
neo^r
PROLONGED COLCEMID ARREST
CYTOCHALASIN B ENUCLEATION
MICRONUCLEATE CELLS
neo^r
neo
MICROCELLS
FUSION PEG/PHA-P
neo^r
MICROCELL HETEROKARYON
RECIPIENT CELL
SELECTION IN G418
neo^r
MICROCELL HYBRID

FIG.2

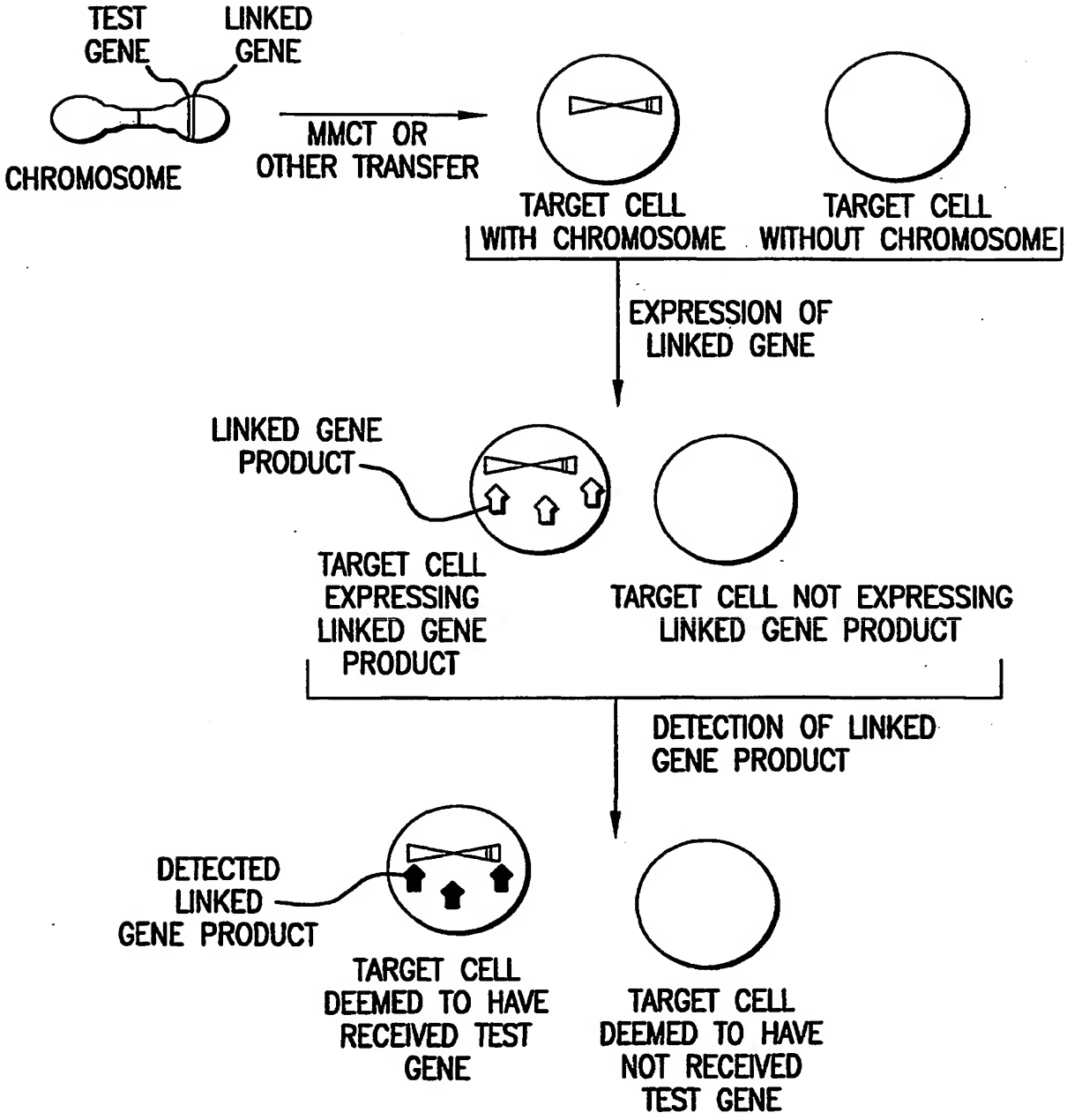
TEST GENE
LINKED GENE
CHROMOSOME
MMCT OR OTHER TRANSFER
TARGET CELL WITH CHROMOSOME
TARGET CELL WITHOUT CHROMOSOME
EXPRESSION OF LINKED GENE
LINKED GENE PRODUCT
TARGET CELL EXPRESSING LINKED GENE PRODUCT
TARGET CELL NOT EXPRESSING LINKED GENE PRODUCT
DETECTION OF LINKED GENE PRODUCT
DETECTED LINKED GENE PRODUCT
TARGET CELL DEEMED TO HAVE RECEIVED TEST GENE
TARGET CELL DEEMED TO HAVE NOT RECEIVED TEST GENE

FIG.3

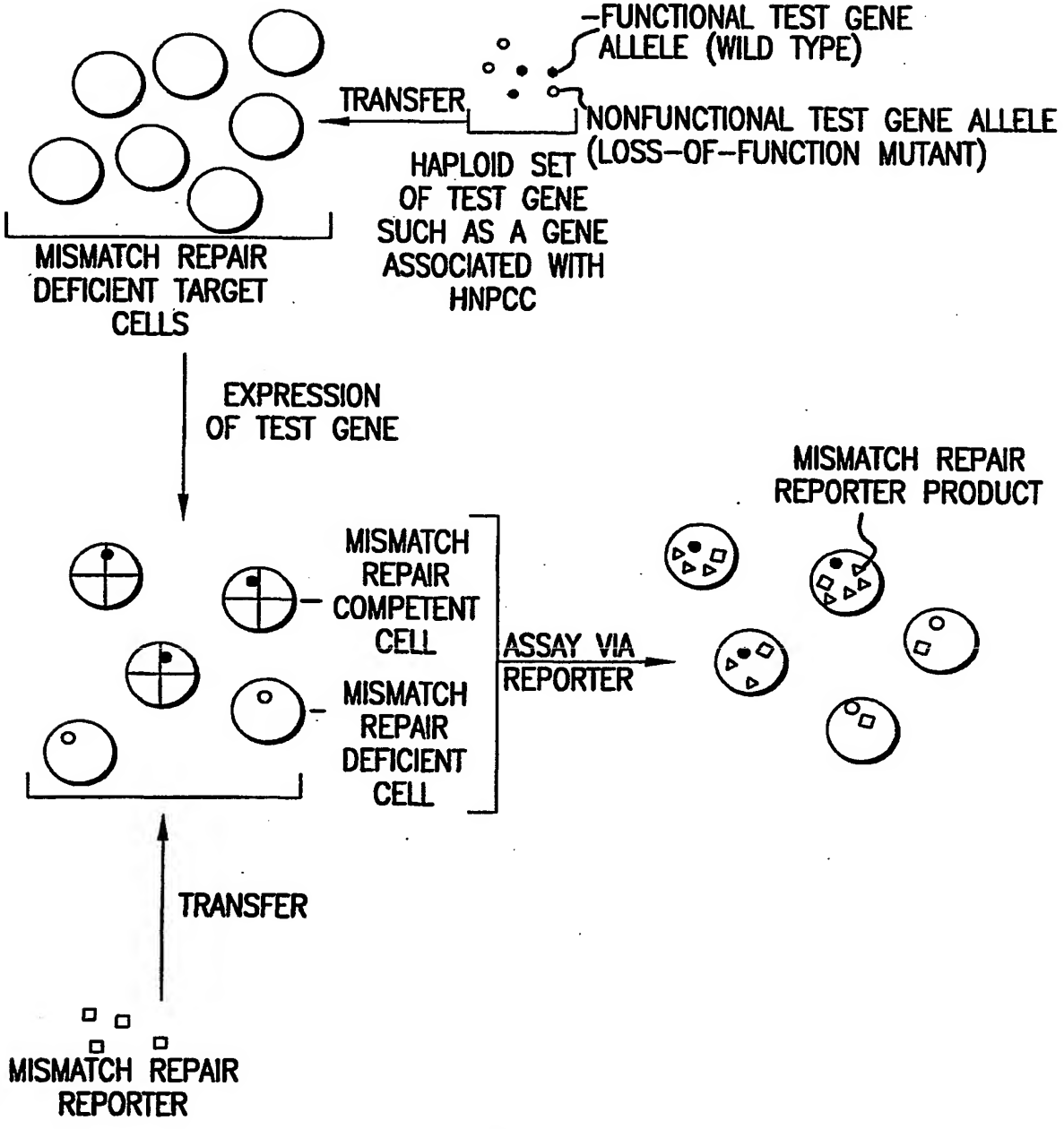
FUNCTIONAL TEST GENE ALLELE (WILD TYPE)
TRANSFER
NONFUNCTIONAL TEST GENE ALLELE (LOSS-OF-FUNCTION MUTANT)
HAPLOID SET OF TEST GENE SUCH AS A GENE ASSOCIATED WITH HNPCC
MISMATCH REPAIR DEFICIENT TARGET CELLS
EXPRESSION OF TEST GENE
MISMATCH REPAIR REPORTER PRODUCT
MISMATCH REPAIR COMPETENT CELL
MISMATCH REPAIR DEFICIENT CELL
ASSAY VIA REPORTER
TRANSFER
MISMATCH REPAIR REPORTER

FIG.4

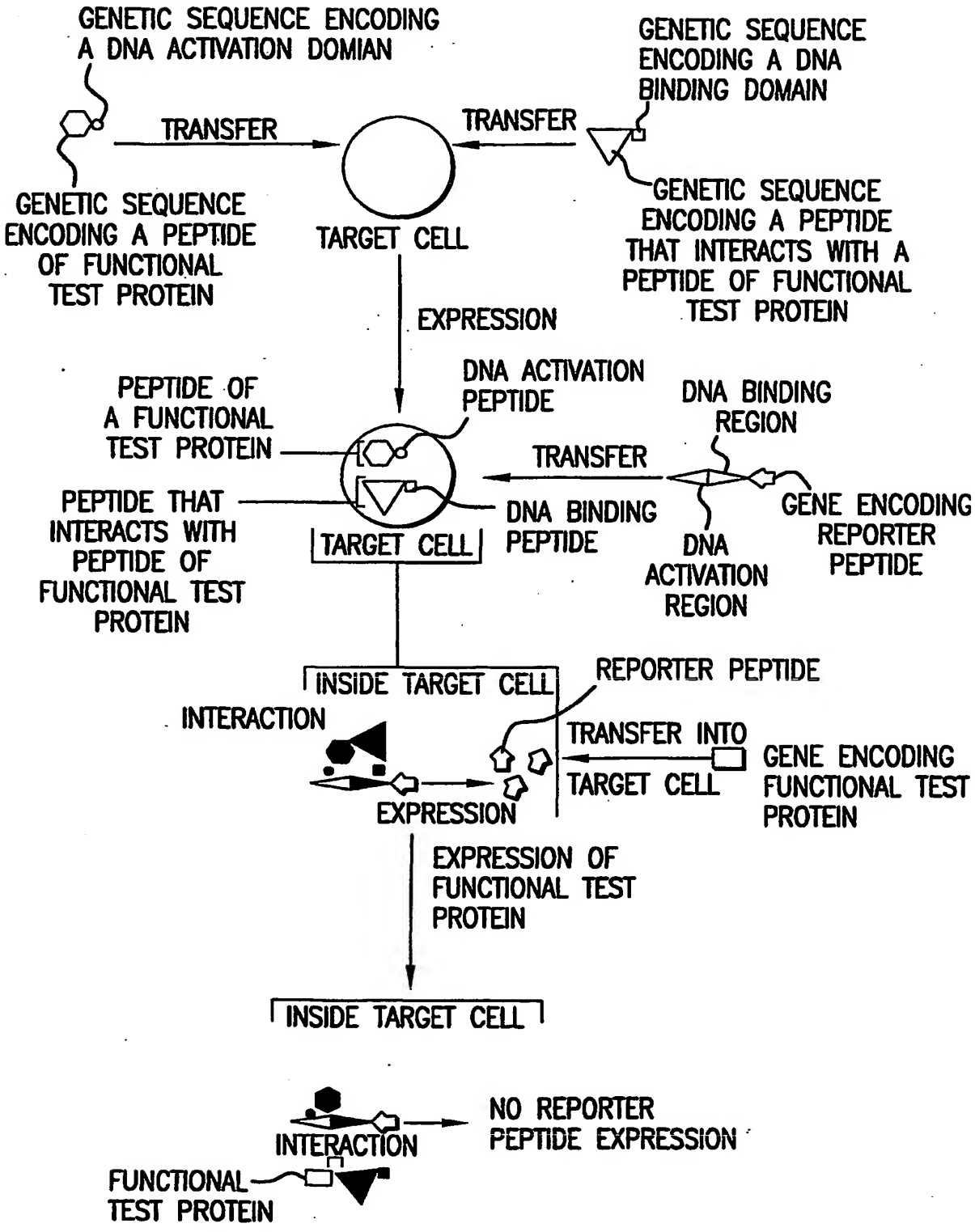
GENETIC SEQUENCE ENCODING A DNA ACTIVATION DOMIAN
GENETIC SEQUENCE ENCODING A DNA BINDING DOMAIN
TRANSFER
TRANSFER
GENETIC SEQUENCE ENCODING A PEPTIDE OF FUNCTIONAL TEST PROTEIN
TARGET CELL
GENETIC SEQUENCE ENCODING A PEPTIDE THAT INTERACTS WITH A PEPTIDE OF FUNCTIONAL TEST PROTEIN
EXPRESSION
PEPTIDE OF A FUNCTIONAL TEST PROTEIN
DNA ACTIVATION PEPTIDE
DNA BINDING REGION
TRANSFER
PEPTIDE THAT INTERACTS WITH PEPTIDE OF FUNCTIONAL TEST PROTEIN
DNA BINDING PEPTIDE
GENE ENCODING REPORTER PEPTIDE
TARGET CELL
DNA ACTIVATION REGION
INSIDE TARGET CELL
REPORTER PEPTIDE
INTERACTION
TRANSFER INTO TARGET CELL
GENE ENCODING FUNCTIONAL TEST PROTEIN
EXPRESSION
EXPRESSION OF FUNCTIONAL TEST PROTEIN
INSIDE TARGET CELL
INTERACTION
NO REPORTER PEPTIDE EXPRESSION
FUNCTIONAL TEST PROTEIN

FIG.5

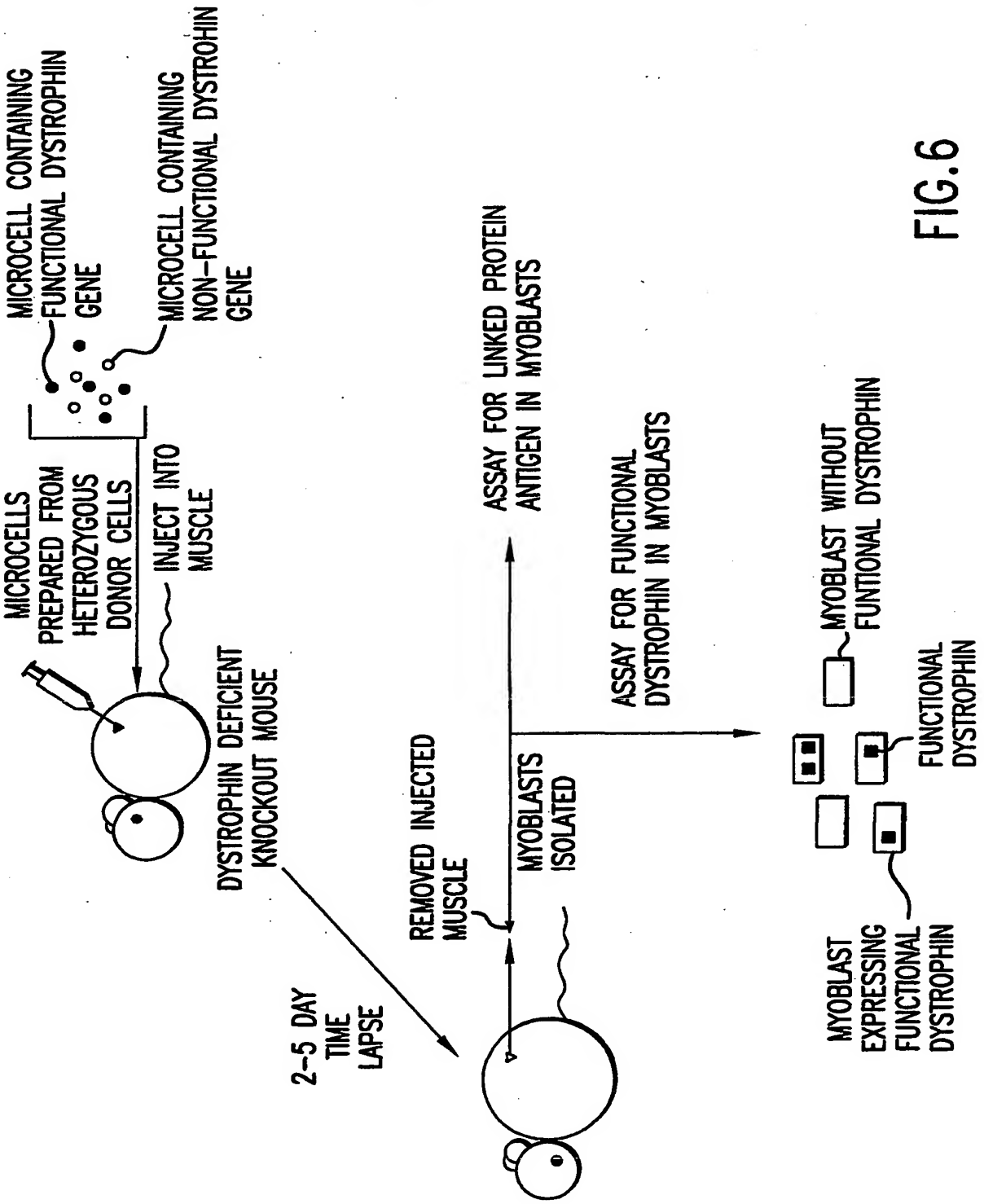
MICROCELL CONTAINING FUNCTIONAL DYSTROPHIN GENE
MICROCELL CONTAINING NON-FUNCTIONAL DYSTROHIN GENE
MICROCELLS PREPARED FROM HETEROZYGOUS DONOR CELLS
INJECT INTO MUSCLE
DYSTROPHIN DEFICIENT KNOCKOUT MOUSE
2-5 DAY TIME LAPSE
REMOVED INJECTED MUSCLE
MYOBLASTS ISOLATED
ASSAY FOR LINKED PROTEIN ANTIGEN IN MYOBLASTS
ASSAY FOR FUNCTIONAL DYSTROPHIN IN MYOBLASTS
MYOBLAST WITHOUT FUNTIONAL DYSTROPHIN
FUNCTIONAL DYSTROPHIN
MYOBLAST EXPRESSING FUNCTIONAL DYSTROPHIN

FIG.6

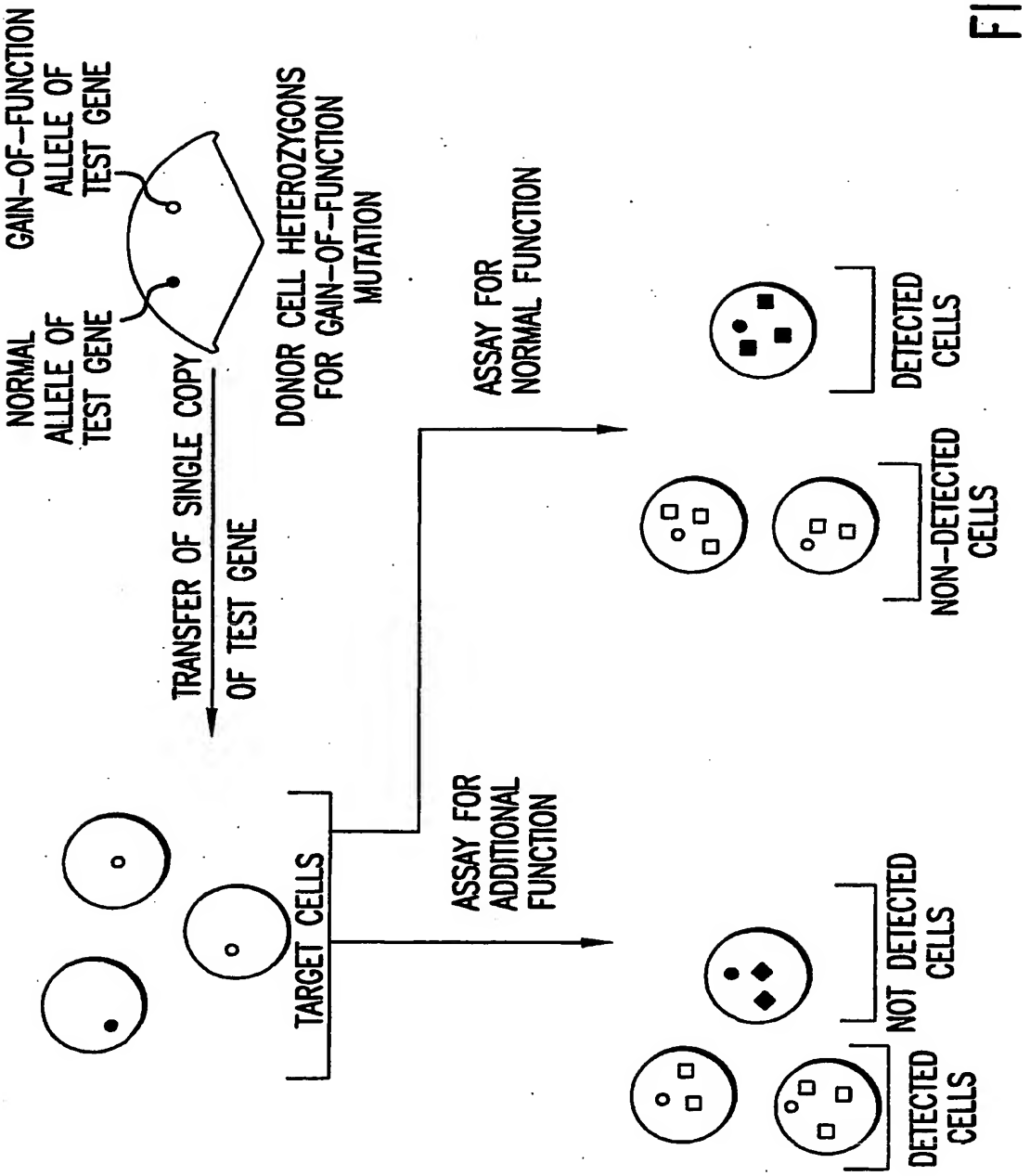
GAIN-OF-FUNCTION ALLELE OF TEST GENE
NORMAL ALLELE OF TEST GENE
DONOR CELL HETEROZYGONS FOR GAIN-OF-FUNCTION MUTATION
TRANSFER OF SINGLE COPY OF TEST GENE
TARGET CELLS
ASSAY FOR NORMAL FUNCTION
ASSAY FOR ADDITIONAL FUNCTION
DETECTED CELLS
NON-DETECTED CELLS
NOT DETECTED CELLS
DETECTED CELLS

FIG.7

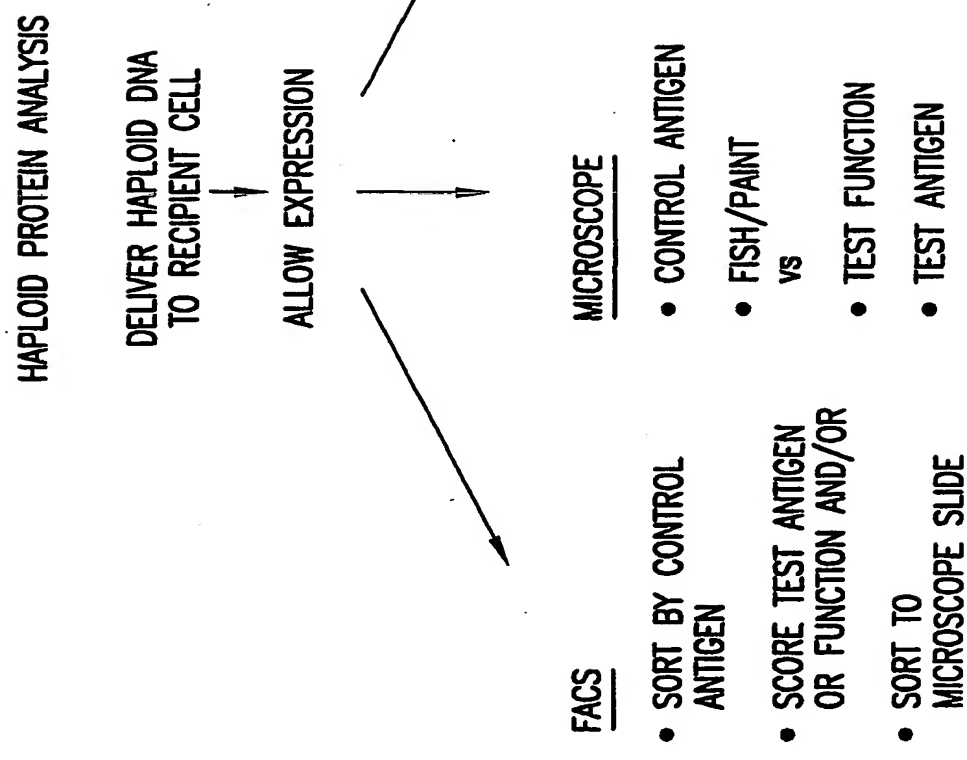
HAPLOID PROTEIN ANALYSIS
DELIVER HAPLOID DNA TO RECIPIENT CELL
ALLOW EXPRESSION
FACS
• SORT BY CONTROL ANTIGEN
• SCORE TEST ANTIGEN OR FUNCTION AND/OR
• SORT TO MICROSCOPE SLIDE
MICROSCOPE
• CONTROL ANTIGEN
• FISH/PAINT
vs
• TEST FUNCTION
• TEST ANTIGEN
TEST FUNCTION
• COMPLEMENTATION
• TWO-HYBRID INHIBITION

FIG.8

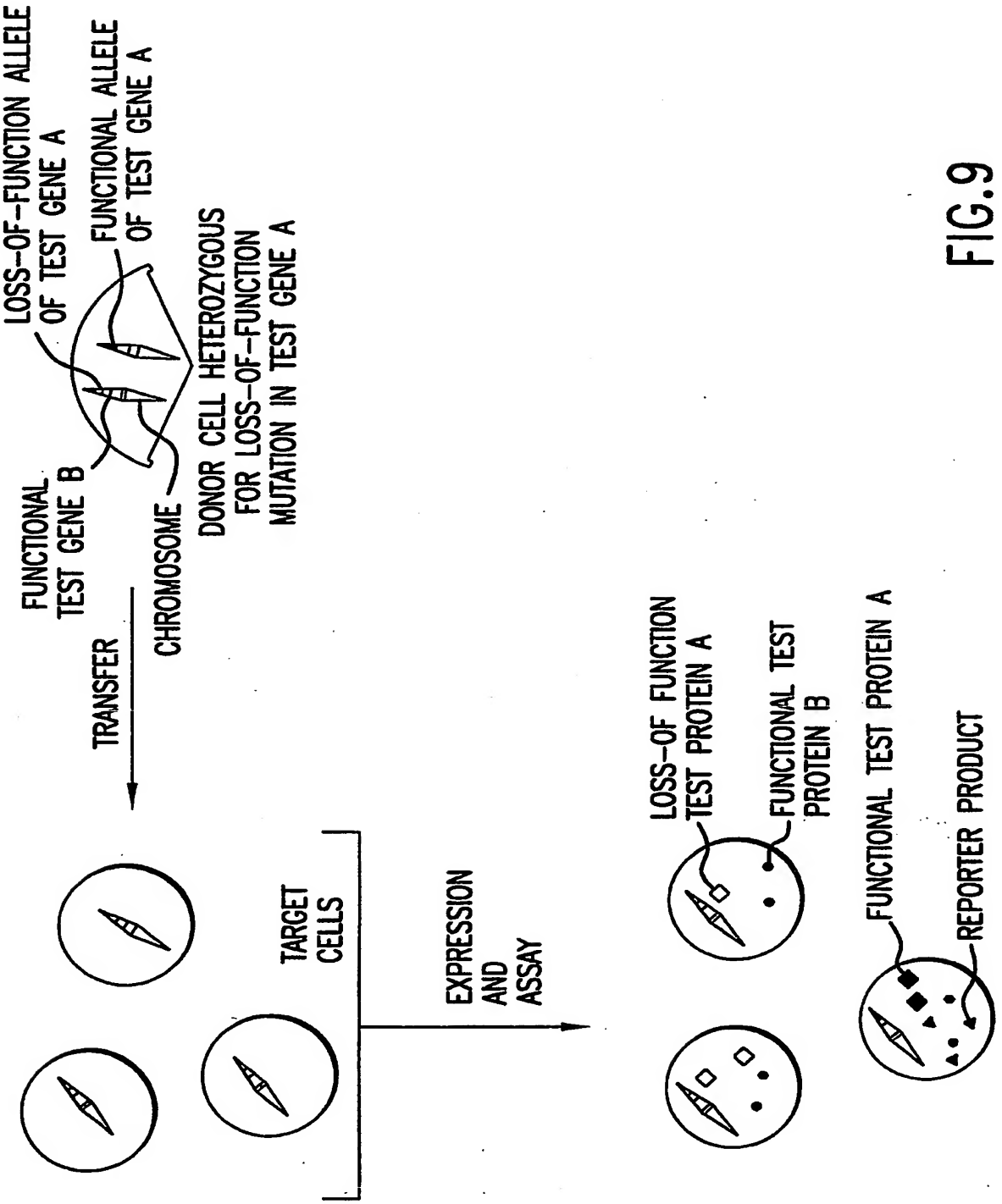
LOSS-OF-FUNCTION ALLELE OF TEST GENE A
FUNCTIONAL ALLELE OF TEST GENE A
FUNCTIONAL TEST GENE B
DONOR CELL HETEROZYGOUS FOR LOSS-OF-FUNCTION MUTATION IN TEST GENE A
CHROMOSOME
TRANSFER
TARGET CELLS
EXPRESSION AND ASSAY
LOSS-OF FUNCTION TEST PROTEIN A
FUNCTIONAL TEST PROTEIN B
FUNCTIONAL TEST PROTEIN A
REPORTER PRODUCT

FIG.9

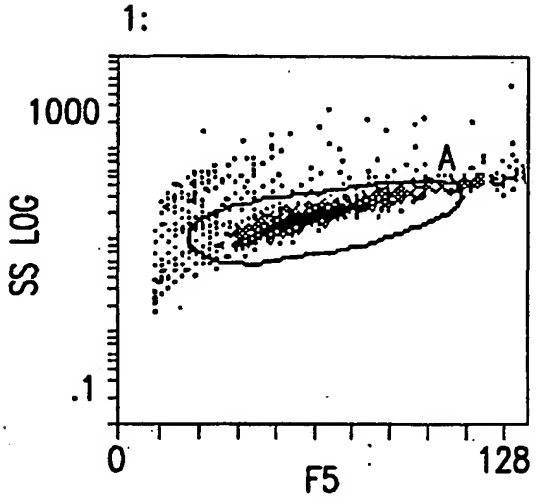

FIG.10A

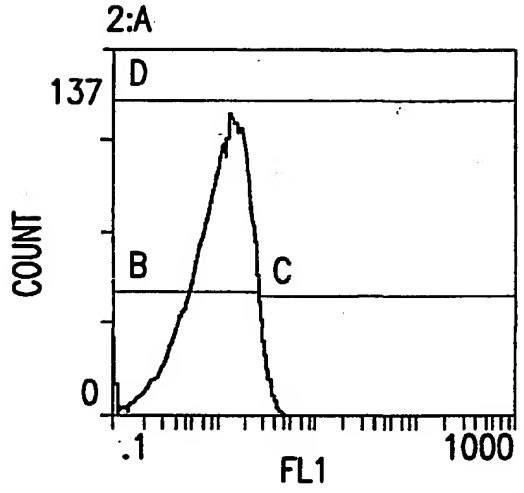

FIG.10B

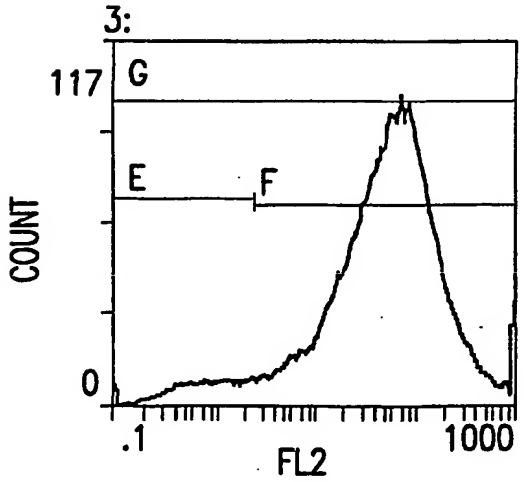

FIG.10C

STATS: NOT NORMALIZED, LISTGATING: DISABLED

HIST	REGION	ID	%	COUNT	PkPosX	PkPosY	PkCnt	FPCVX	FPCVY	Mnl X	Mn
1	A'	A	69.2	20000	59.0	18.2	92	28.59	34.79	61.1	21.8

HIST	REGION	ID	%	COUNT	PkPosX	PkCnt	HPCV	Min	Max	Mn
2	B	B	94.7	18933	0.102	184	0.00	0.102	2.75	1.18
	C	C	5.6	1110	2.78	47	86.40	2.75	996.7	3.99
	D	D	100	20000	0.102	184	0.00	0.102	1024.0	1.34
3	E	E	7.1	2044	0.102	68	0.00	0.102	2.43	0.970
	F	F	92.9	26858	1014.8	252	**	2.43	1024.0	111.8
	G	G	100	28896	1014.8	252	**	0.102	1024.0	104.0

FIG.10D

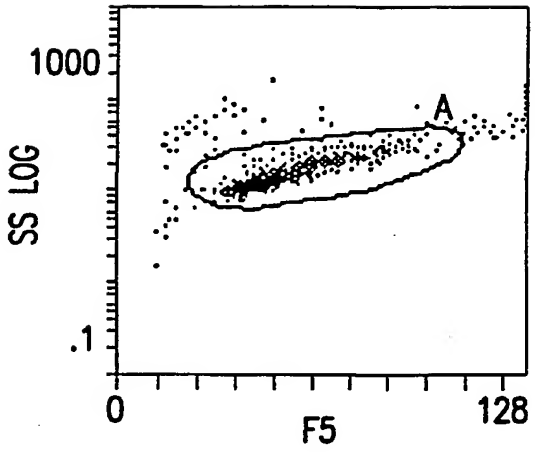

FIG.11A

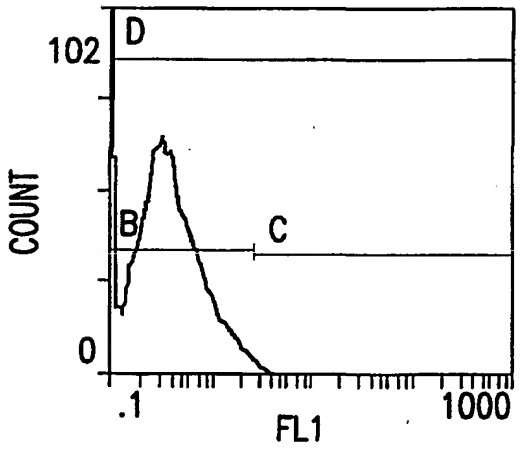

FIG.11B

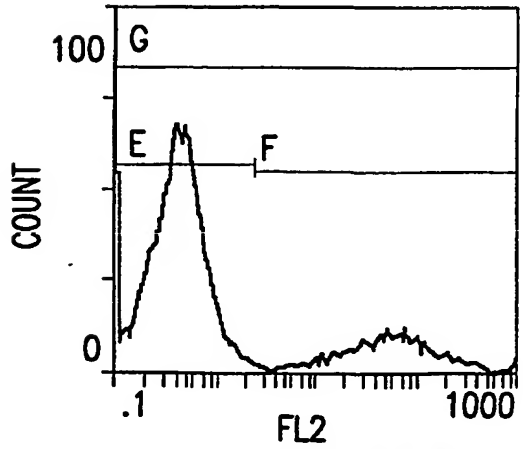

FIG.11C

STATS: NOT NORMALIZED, LISTGATING: DISABLED

HIST	REGION ID		%	COUNT	PkPosX	PkPosY	PkCnt	FPCVX	FPCVY	Mnl X	MnlY
1	A'	A	89.6	12866	45.0	11.8	111	29.34	36.25	53.4	16.3

HIST	REGION ID		%	COUNT	PkPosX	PkPosY	PkCnt	HPCV	Min	Max	Mn
2	B	B	99.0	12742	0.102		1011	0.00	0.102	2.75	0.486
	C	C	1.0	124	2.88		8	0.95	2.75	996.7	3.56
	D	D	100	12866	0.102		1011	0.00	0.102	1024.0	0.516
3	E	E	78.9	11331	0.102		823	0.00	0.102	2.43	0.498
	F	F	21.1	3030	1014.8		21	* *	2.43	1024.0	95.3
	G	G	100	14358	0.102		823	0.00	0.102	1024.0	20.5

FIG.11D

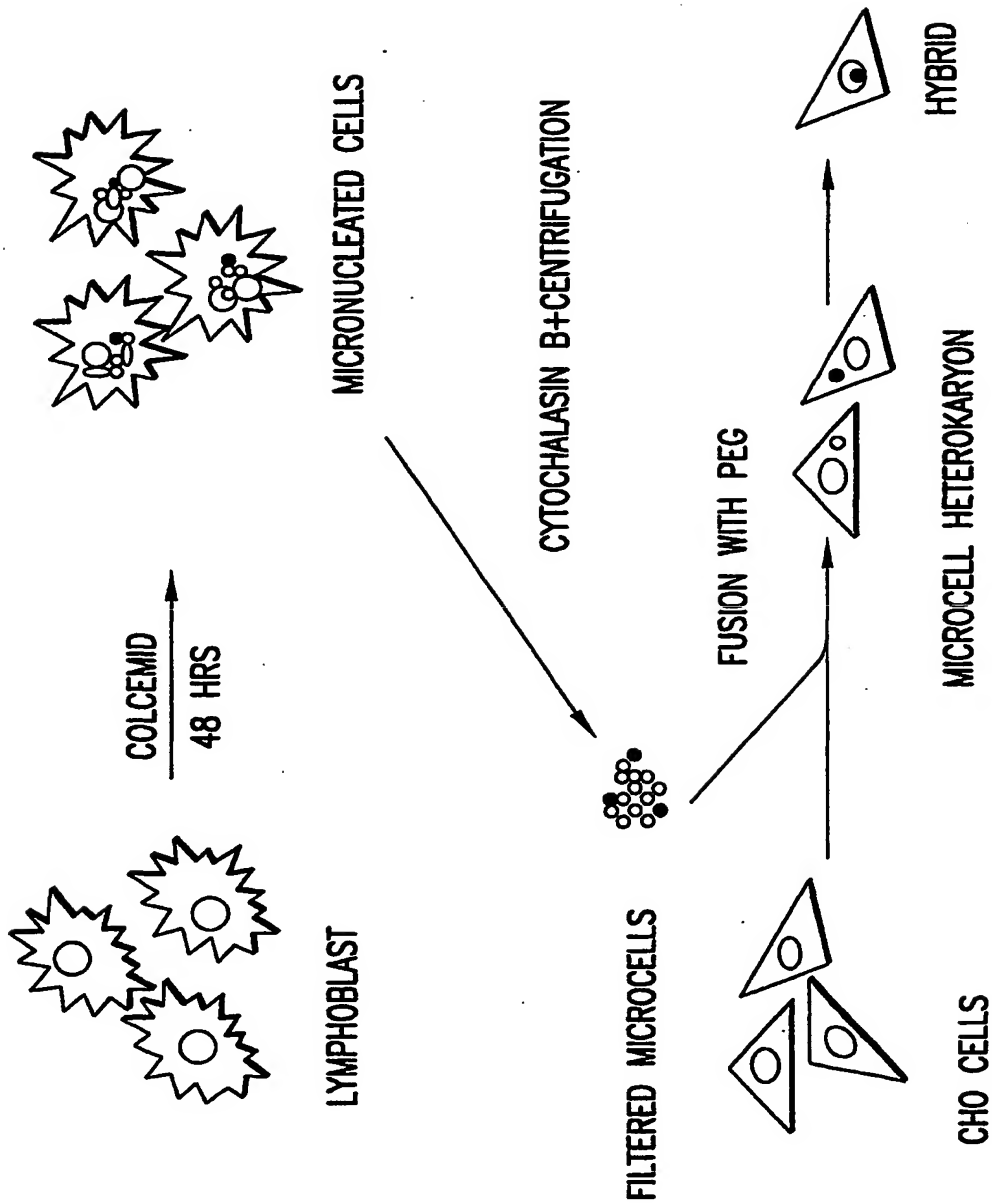
LYMPHOBLAST
COLCEMID
48 HRS
MICRONUCLEATED CELLS
CYTOCHALASIN B+CENTRIFUGATION
FILTERED MICROCELLS
FUSION WITH PEG
CHO CELLS
MICROCELL HETEROKARYON
HYBRID

FIG.12

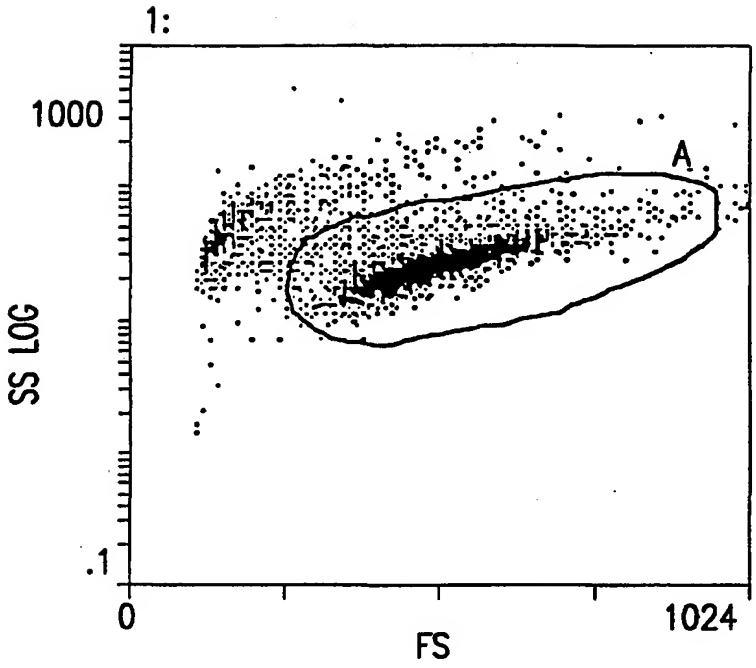

FIG.13A1

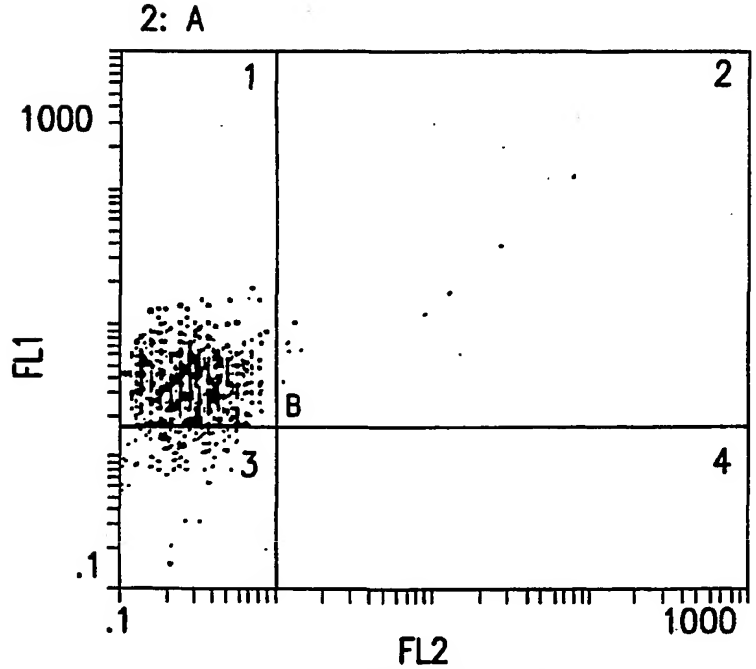

FIG.13A2

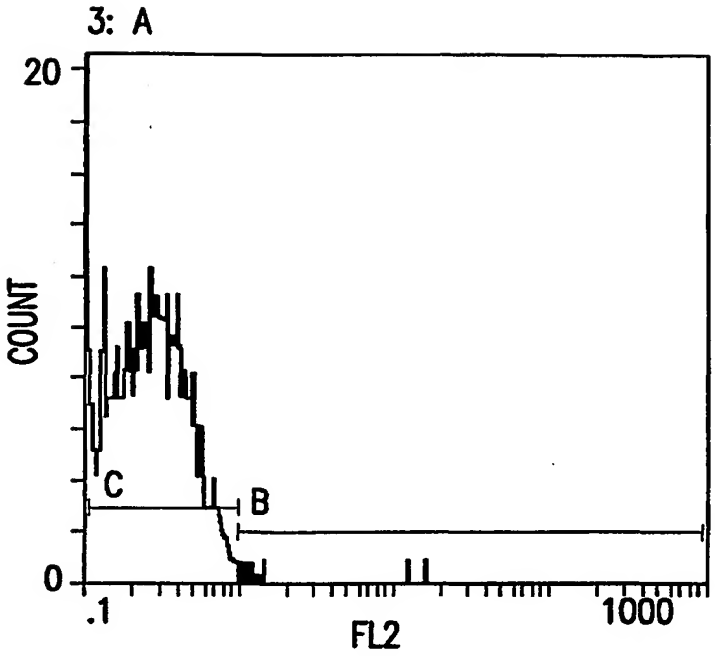

FIG.13A3

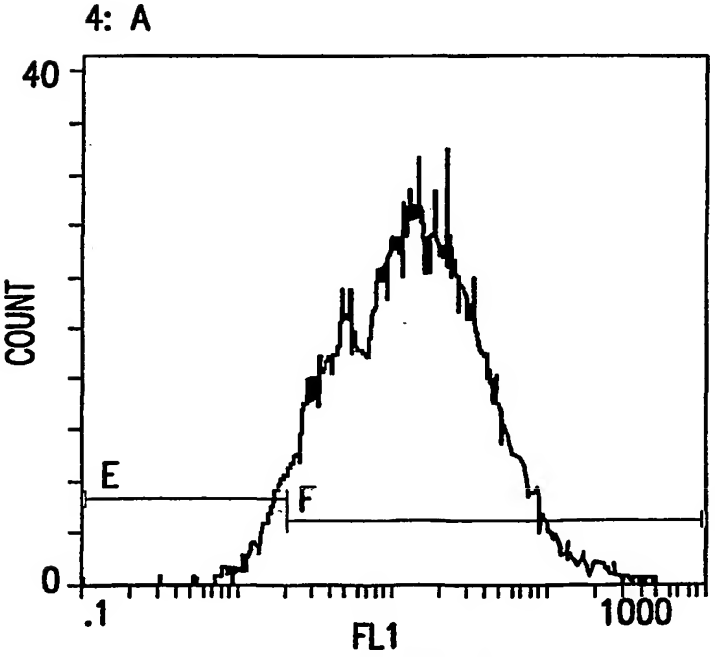

FIG.13A4

STATS: NORMALIZED, LISTGATING: DISABLED

HIST	REGION	ID	%	COUNT	PkPosX	PkPosY	PkCnt	FPCVX	FPCVY	Mnl X	MnlY
1	A	A	76.8	10000	464.0	21.0	56	23.20	38.40	500.9	27.7
2	B1	B	96.8	9677	0.102	14.7	289	40.65	108.00	0.139	24.1
	B2	B	0.35	35	1.47	5.76	2	139.91	89.48	9.59	14.6
	B3	B	2.87	287	0.102	1.37	11	55.47	50.95	0.273	1.23
	B4	B	0.01	1	1.10	1.58	1	0.00	0.00	1.14	1.63

HIST	REGION	ID	%	COUNT	PkPosX	PkCnt	HPCV	Min	Max	Mn
3	C	C	16.7	1667	0.116	20	0.53	0.110	1.04	0.325
	D	D	0.36	36	1.08	2	0.38	1.02	970.2	9.47
4	E	E	5.09	509	1.84	13	3.59	0.110	2.10	1.53
	F	F	95.2	9519	15.5	47	10.86	2.07	979.0	24.3

FIG.13A5

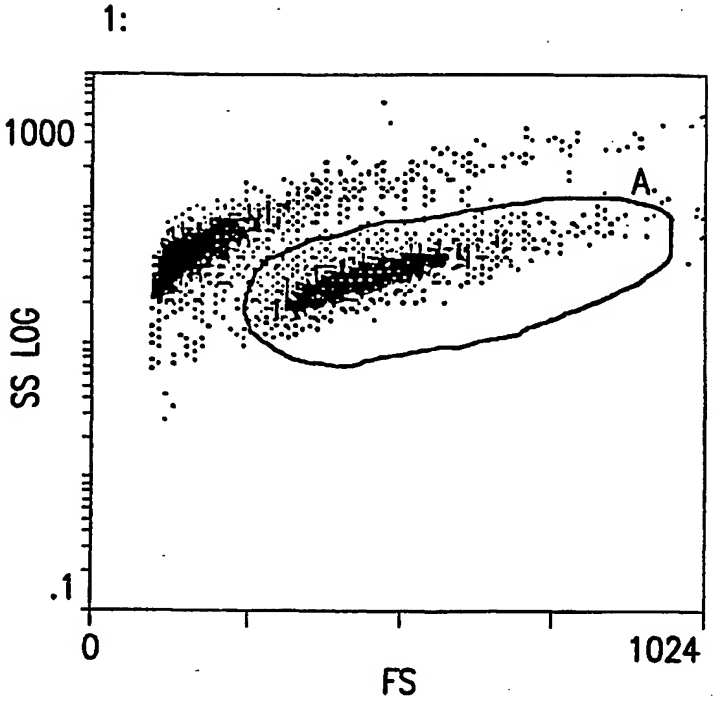

FIG.13B1

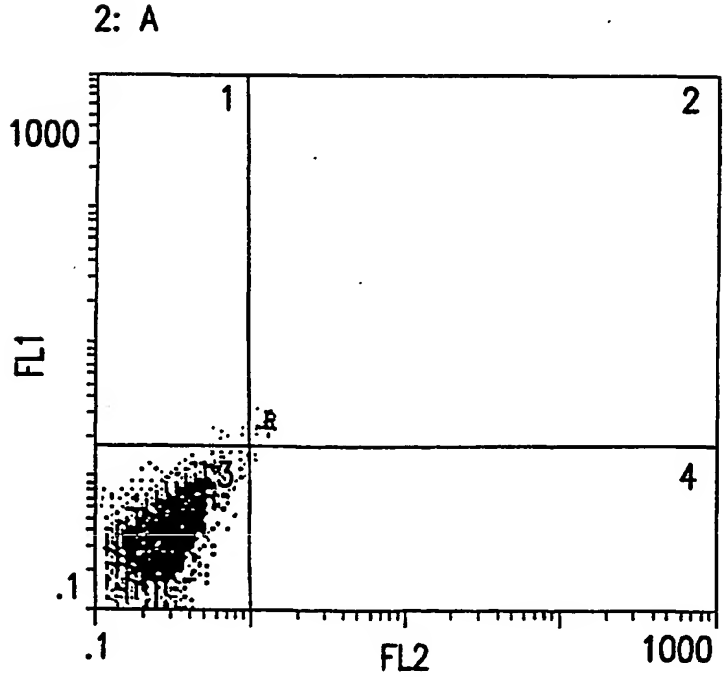

FIG.13B2

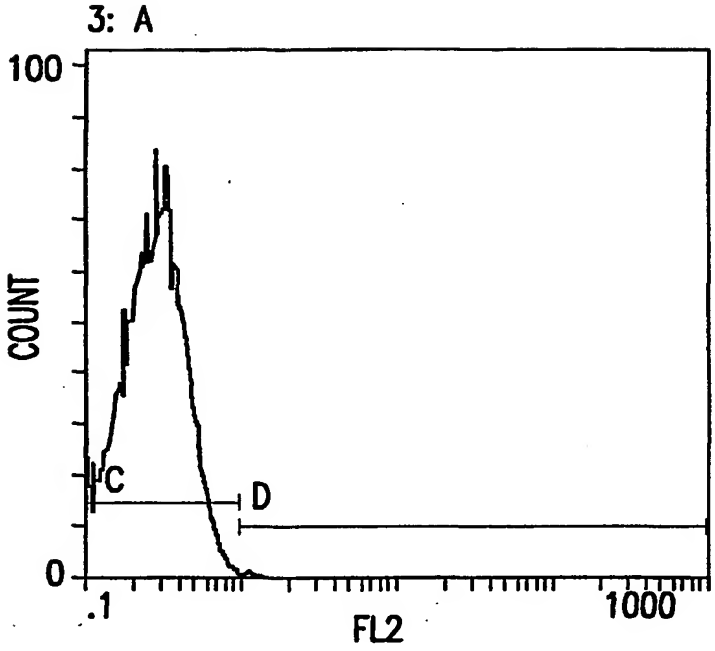

FIG.13B3

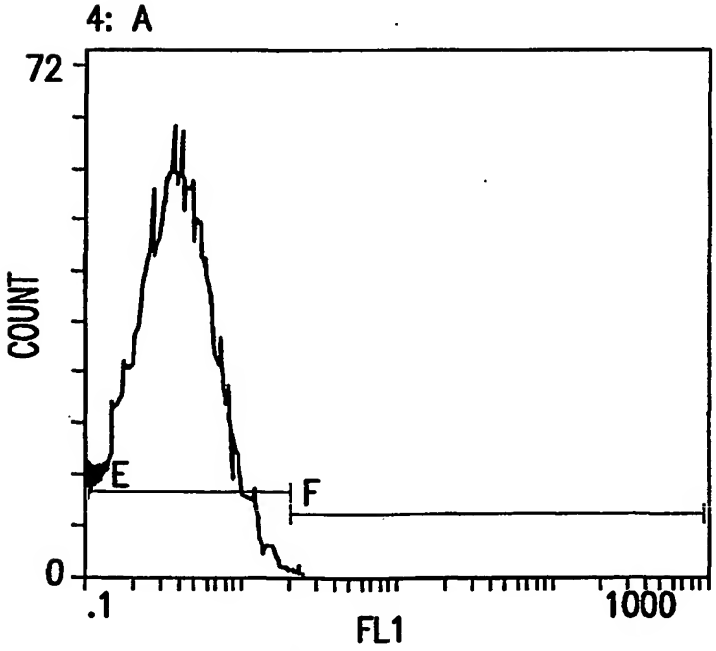

FIG.13B4

STATS: NORMALIZED, LISTGATING: DISABLED

HIST	REGION	ID	%	COUNT	PkPosX	PkPosY	PkCnt	FPCVX	FPCVY	Mnl X	MnlY
1	A	A	45.5	10000	408.0	22.6	65	22.18	34.15	458.5	31.2
2	B1	B	0.37	37	0.665	1.82	3	39.12	29.87	0.764	2.19
	B2	B	0.30	30	1.18	2.26	3	75.00	57.16	2.61	3.48
	B3	B	99.2	9923	0.102	0.102	279	51.58	65.59	0.285	0.398
	B4	B	0.10	10	1.10	1.37	3	5.98	17.11	1.15	1.39

HIST	REGION	ID	%	COUNT	PkPosX	PkCnt	HPCV	Min	Max	Mn
3	C	C	88.6	8888	0.271	100	3.72	0.110	1.04	0.310
	D	D	0.40	40	1.14	4	0.76	1.02	970.2	2.22
4	E	E	90.4	9043	0.398	70	20.55	0.110	2.10	0.435
	F	F	0.30	30	2.14	2	0.57	2.07	979.0	3.91

FIG.13B5

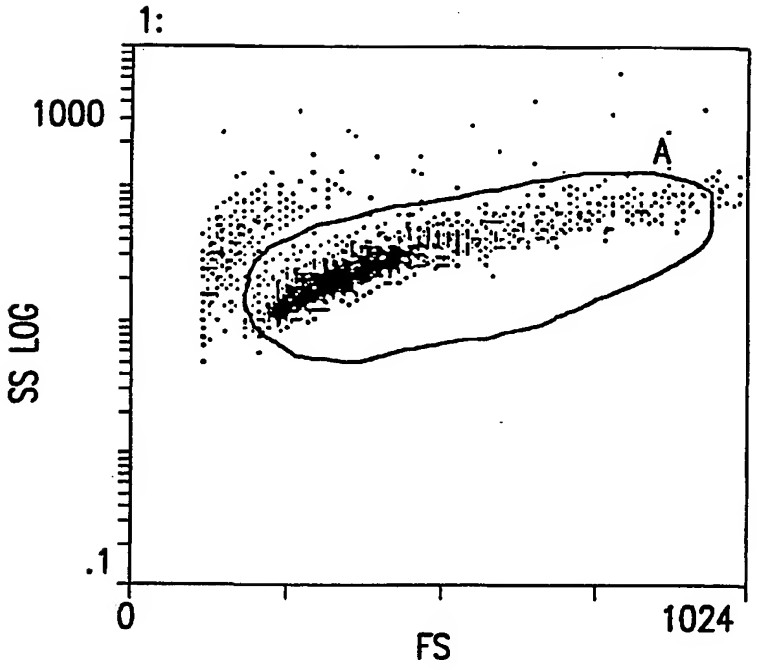

FIG.14A

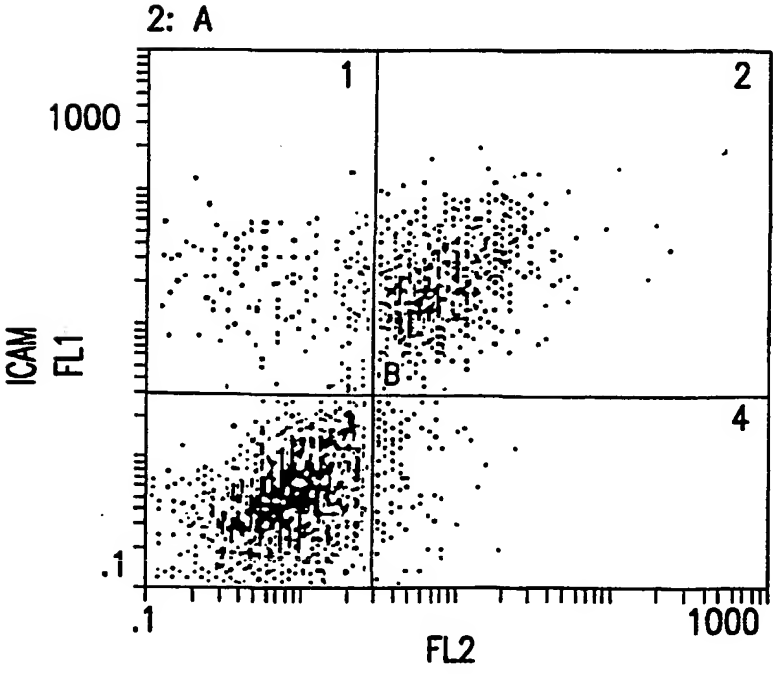

FIG.14B

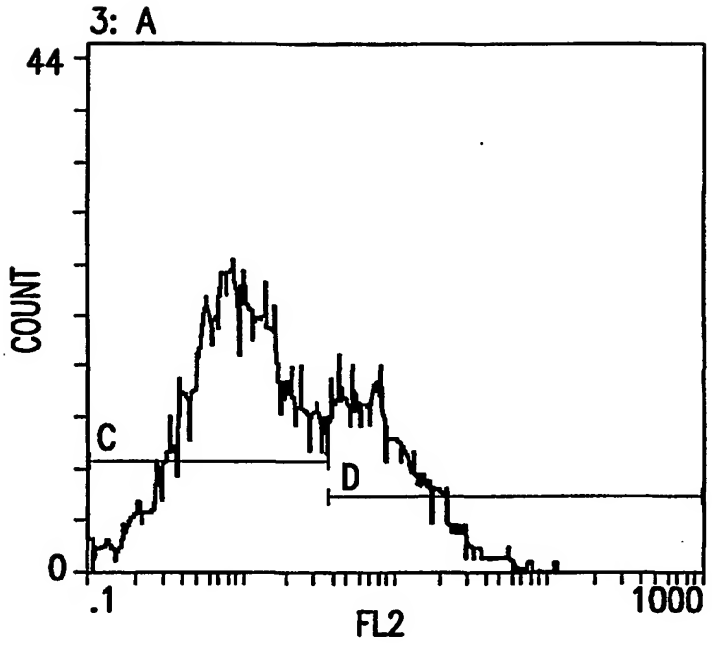

FIG.14C

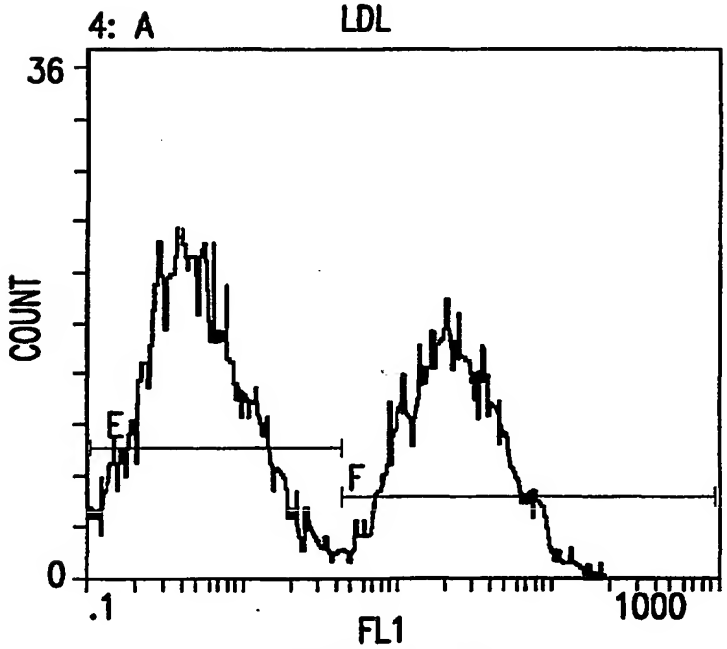

FIG.14D

STATS: NORMALIZED, LISTGATING: DISABLED

HIST	REGION	ID	%	COUNT	PkPosX	PkPosY	PkCnt	FPCVX	FPCVY	Mnl X	MnlY
1	A	A	84.0	8572	264.0	14.7	48	40.53	49.42	397.2	29.9
2	B1	B	FITC 13.8	1179	0.102	34.8	36	137.10	82.75	0.690	33.2
	B2	B	DOUBLE LABELED 26.2	2247	4.32	8.87	11	69.76	73.66	12.0	27.4
	B3	B	NOT LABELED 54.0	4632	0.102	0.102	28	78.81	82.60	0.998	0.598
	B4	B	Rhod 6.00	514	19.6	0.102	10	82.82	122.34	10.8	0.766

HIST	REGION	ID	%	COUNT	PkPosX	PkCnt	HPCV	Min	Max	Mn
3	C	C	62.4	5351	0.825	41	0.82	0.106	3.88	1.22
	D	D	28.3	2425	7.68	23	3.01	3.88	970.2	1.29
4	E	E	60.9	5222	0.102	826	0.00	0.102	4.32	0.658
	F	F	39.2	3361	18.7	23	1.63	4.17	979.0	29.9

FIG.14E

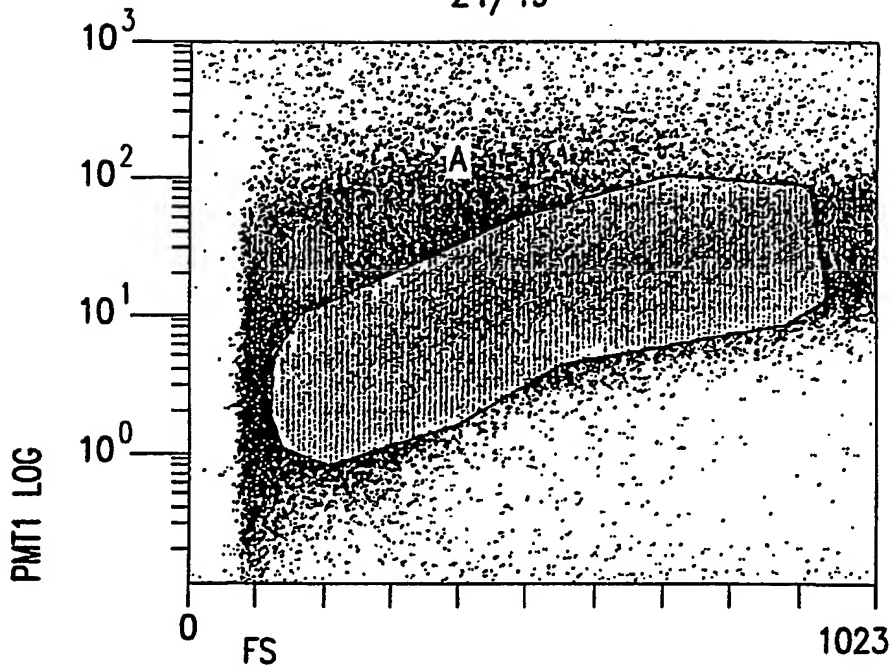

FIG.15A1

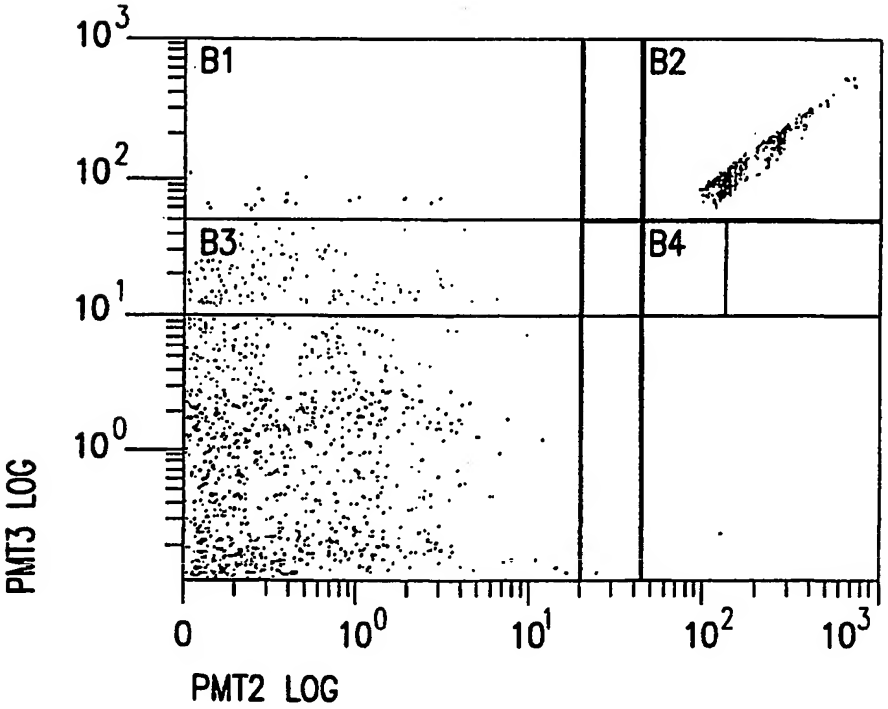

FIG.15A2

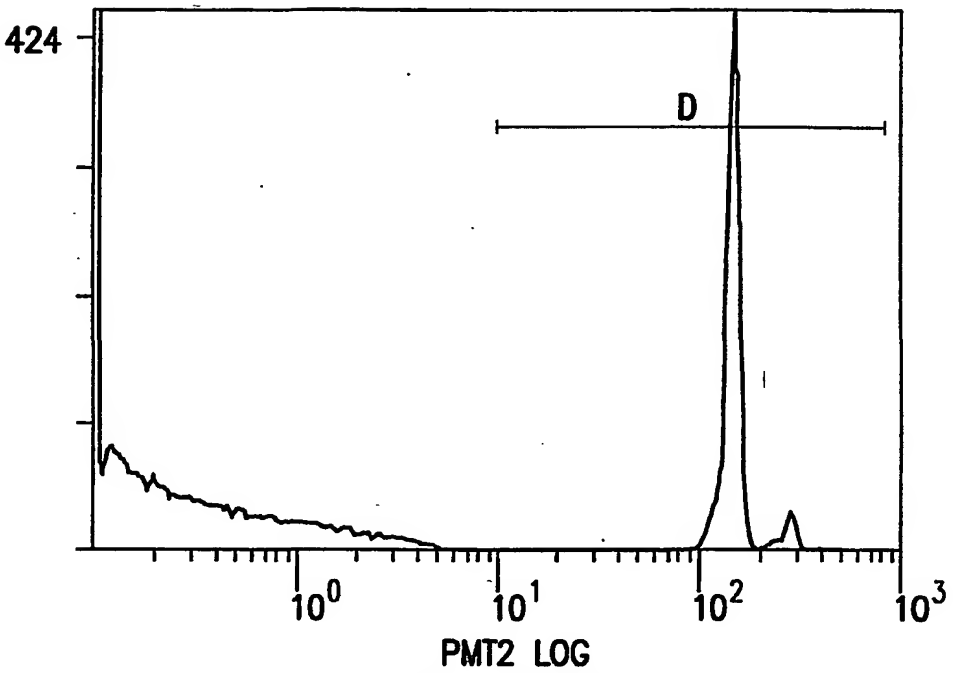

FIG.15A3

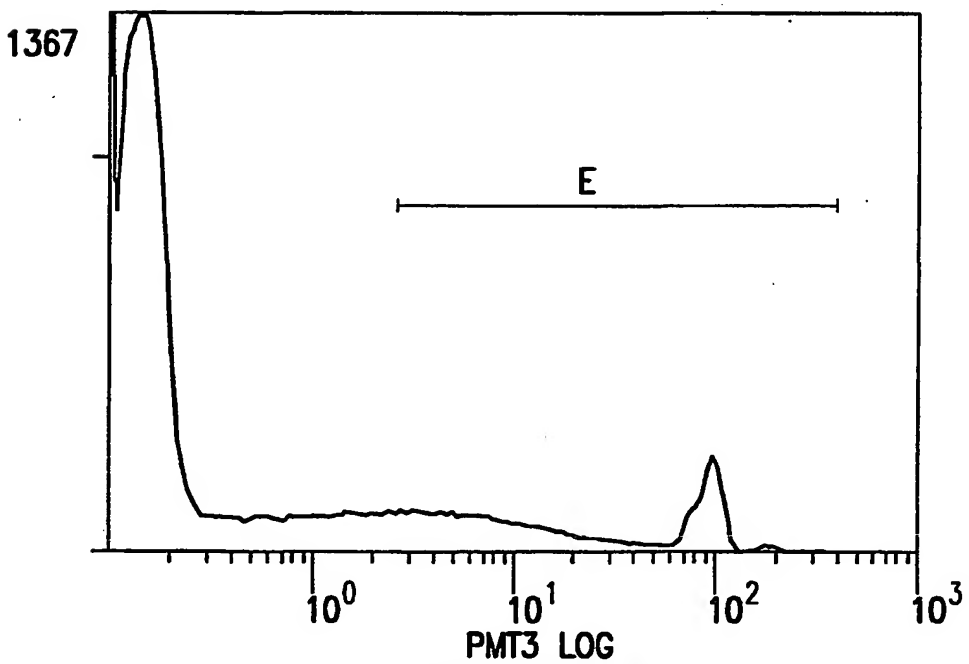

FIG.15A4

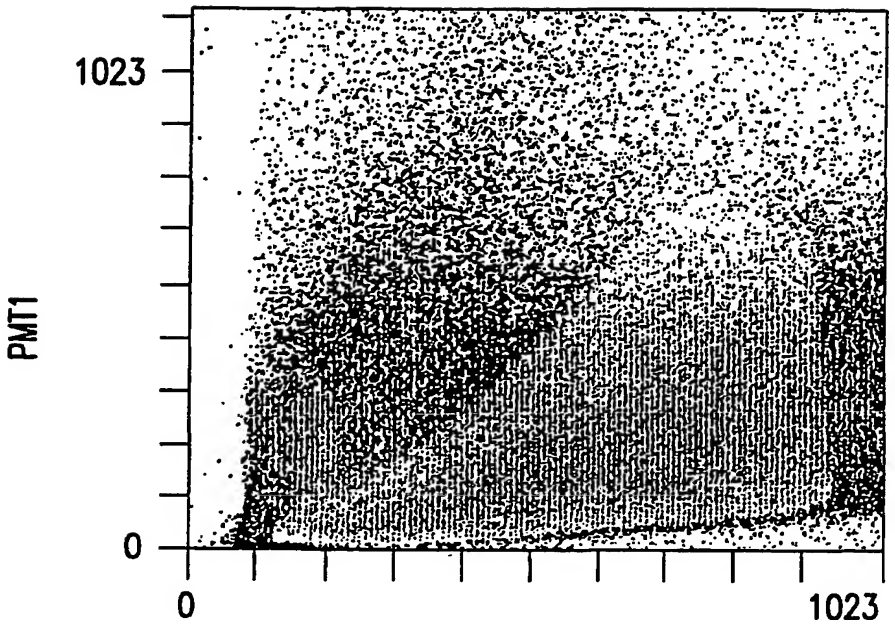

FIG.15A5

GATE:- UNGATED

FILENAME:- 00001264 158.LMD

MEAN CALCULATION METHOD:-LOG-LOG

REGION	NUMBER	%TOTAL	%GATED	X-MEDIAN
A	1475351	79.68	79.68	371.0
B1	1983	0.11	0.11	0.1
B2	9512	0.51	0.51	144.1
B3	1839985	99.38	99.38	0.1
B4	57	0.00	0.00	70.8
C	9565	0.52	0.52	144.1
D	9764	0.53	0.53	144.1
E	34097	1.84	1.84	14.2

FIG.15A6

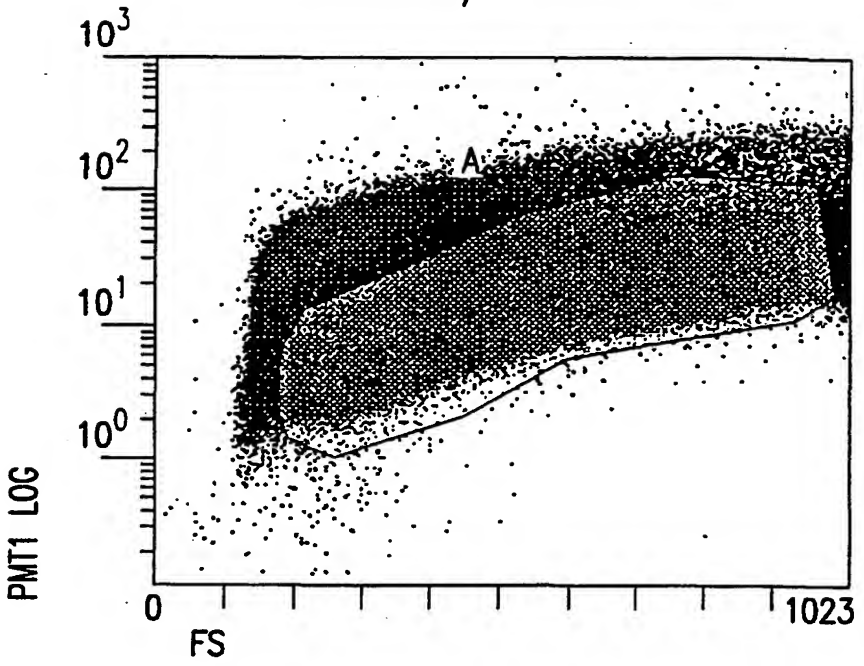

FIG.15B1

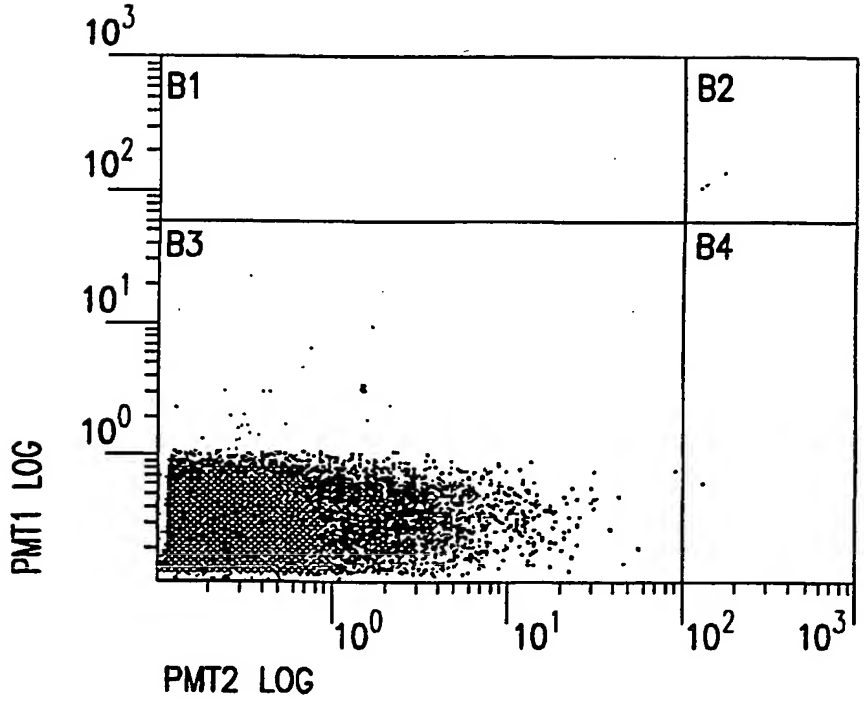

FIG.15B2

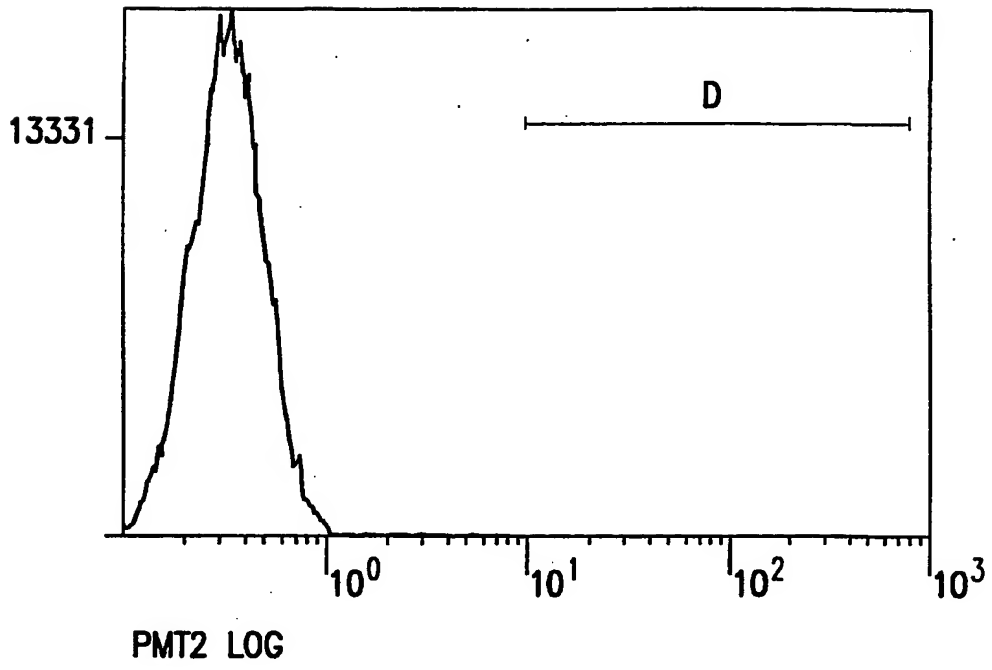

FIG.15B3

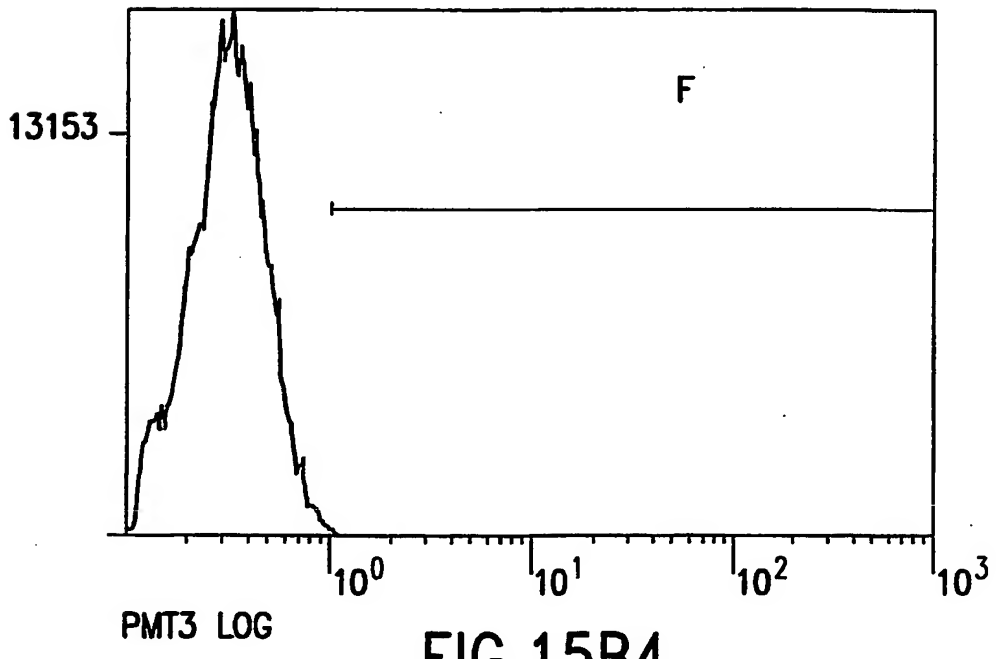

FIG.15B4

GATE:- UNGATED				
FILENAME:- 00001721 566.LMD				
MEAN CALCULATION METHOD:-LOG-LOG				
REGION	NUMBER	%TOTAL	%GATED	X-MEDIAN
A	1172798	83.13	83.13	414.0
B1	1	0.00	0.00	0.7
B2	15	0.00	0.00	159.1
B3	1410858	100.00	100.00	0.3
B4	6	0.00	0.00	172.5
C	21	0.00	0.00	164.9
D	254	0.02	0.02	13.4
F	609	0.04	0.04	1.0

FIG.15B5

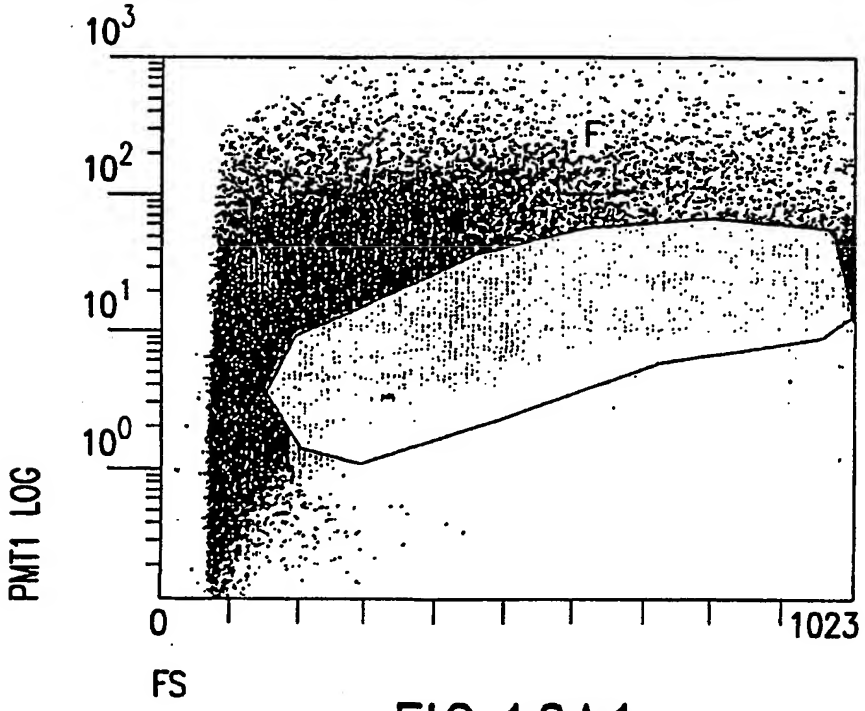

FIG.16A1

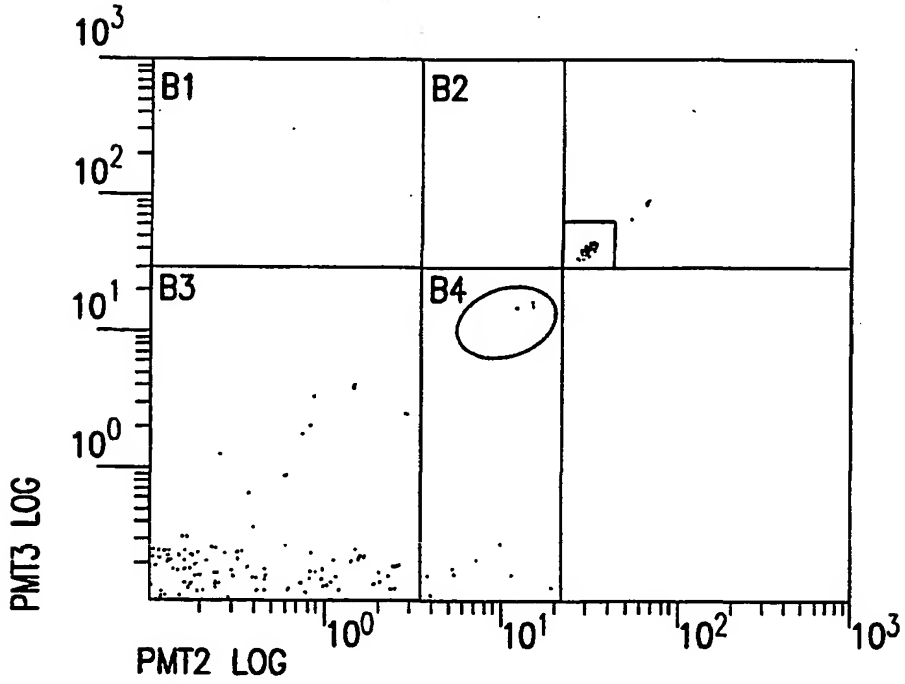

FIG.16A2

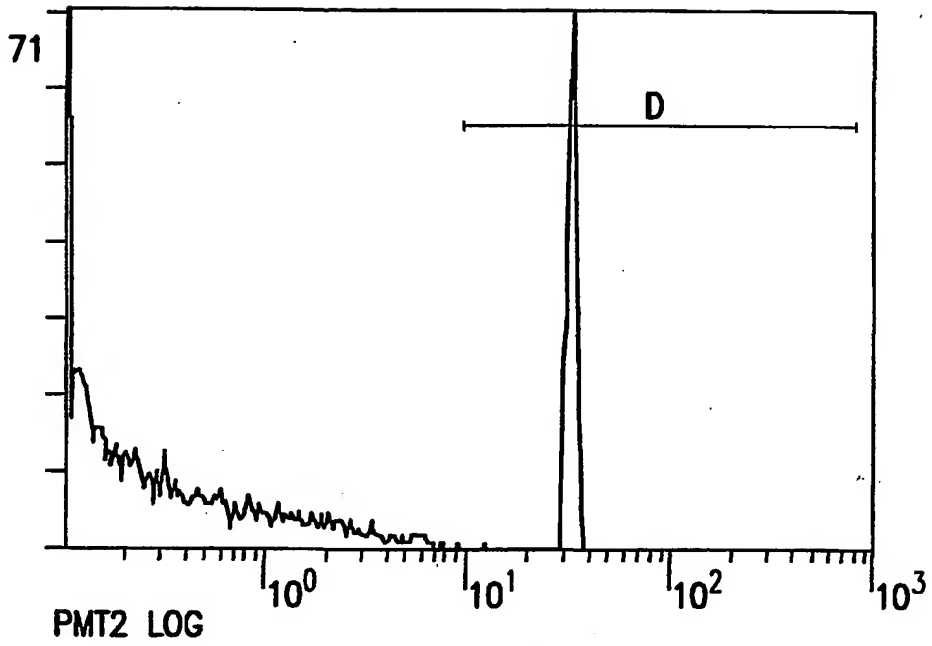

FIG.16A3

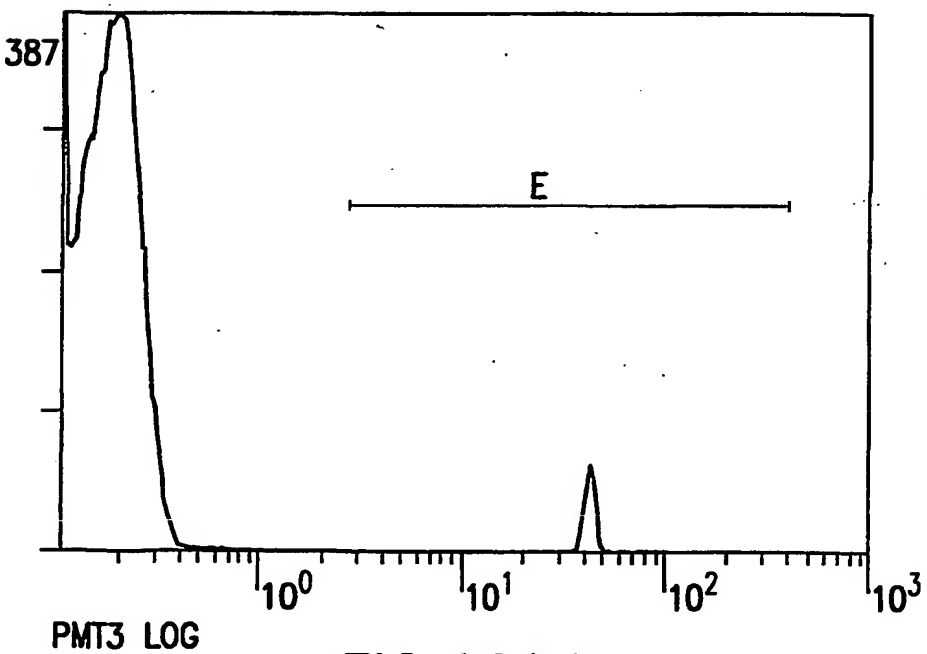

FIG.16A4

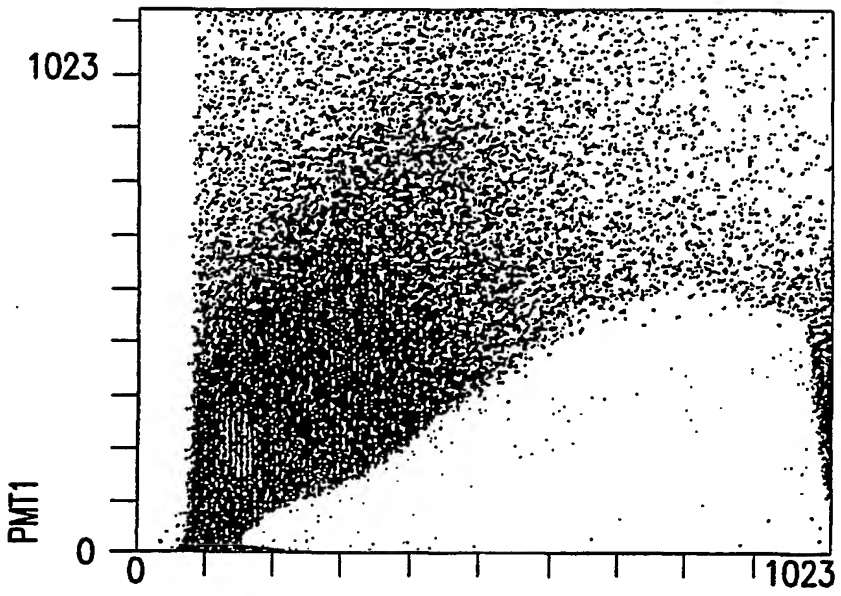

FIG.16A5

GATE:- UNGATED

FILENAME:- 00001224 118.LMD

MEAN CALCULATION METHOD:-LOG-LOG

REGION	NUMBER	%TOTAL	%GATED	X-MEDIAN
B1	203	0.02	0.02	0.1
B2	1029	0.10	0.10	32.7
B3	979209	99.84	99.84	0.1
B4	326	0.03	0.03	5.5
C	1342	0.14	0.14	32.1
D	1082	0.11	0.11	32.7
E	1489	0.15	0.15	41.7
F	574114	58.54	58.54	383.0

FIG.16A6

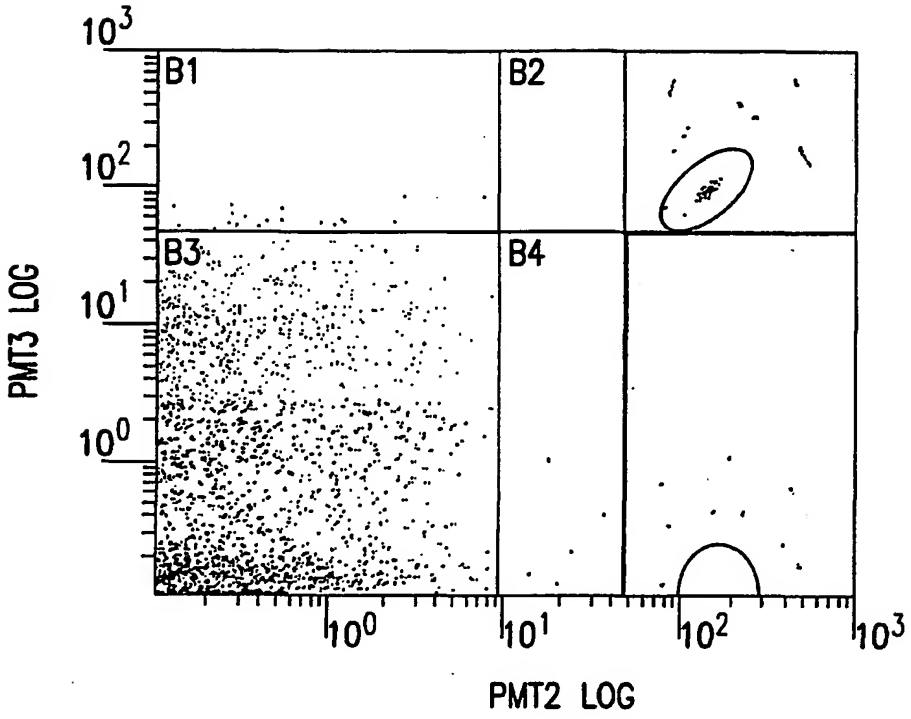

FIG.16B1

GATE:- UNGATED

FILENAME:- FHhetero_7-21_olaf.LMD

MEAN CALCULATION METHOD:-LOG-LOG

REGION	NUMVER	%TOTAL	%GATED	X-MEDIAN
A	3078481	87.70	87.70	371.0
B1	1182	0.03	0.03	0.1
B2	335	0.01	0.01	144.1
B3	3508248	99.95	99.95	0.1
B4	282	0.01	0.01	16.5
C	368	0.01	0.01	144.1
D	587	0.02	0.02	134.1
E	19444	0.55	0.55	7.4

FIG.16B2

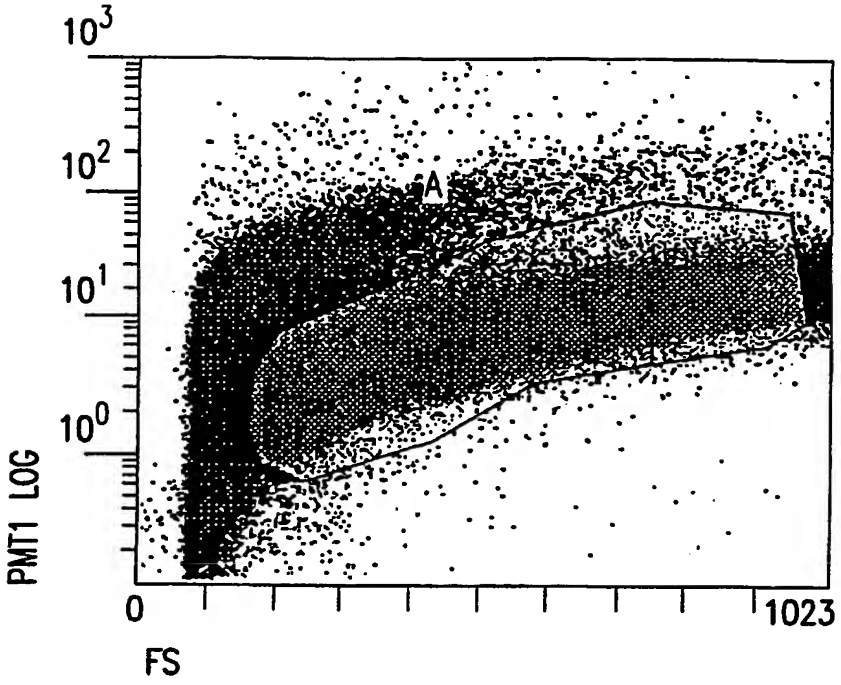

FIG.17A1

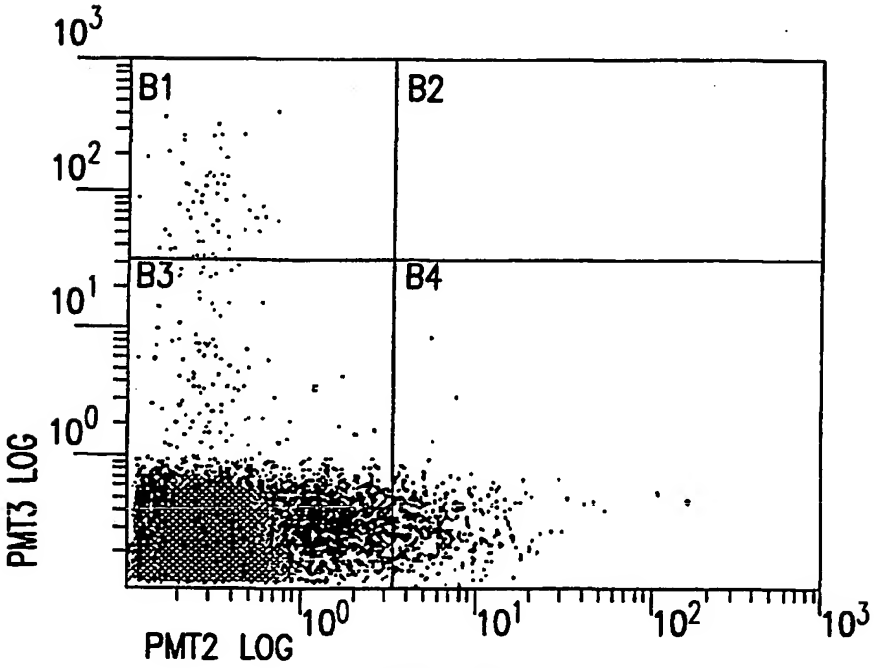

FIG.17A2

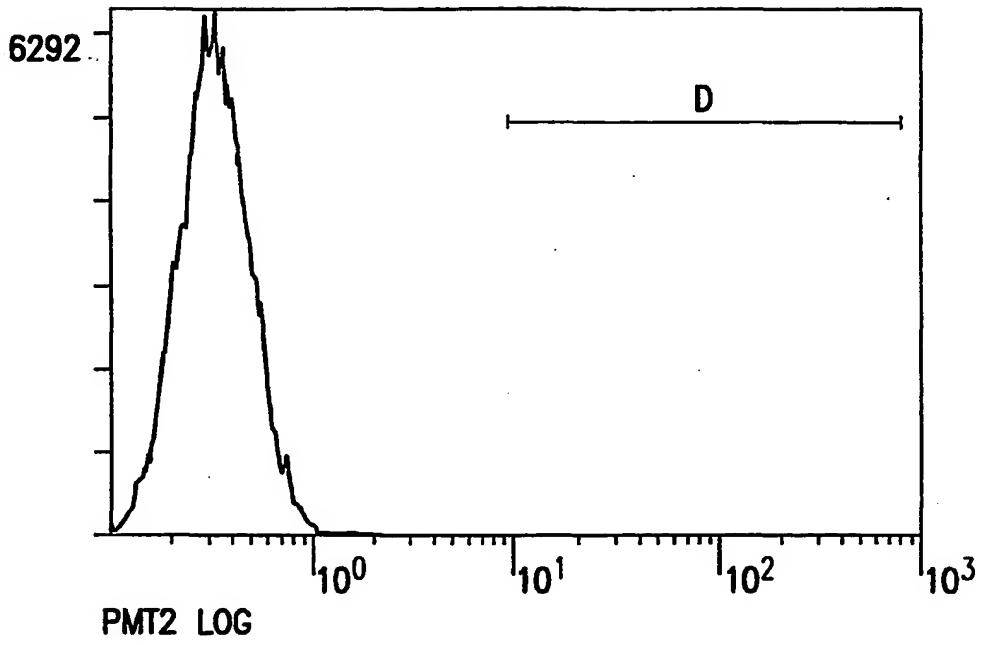

FIG.17A3

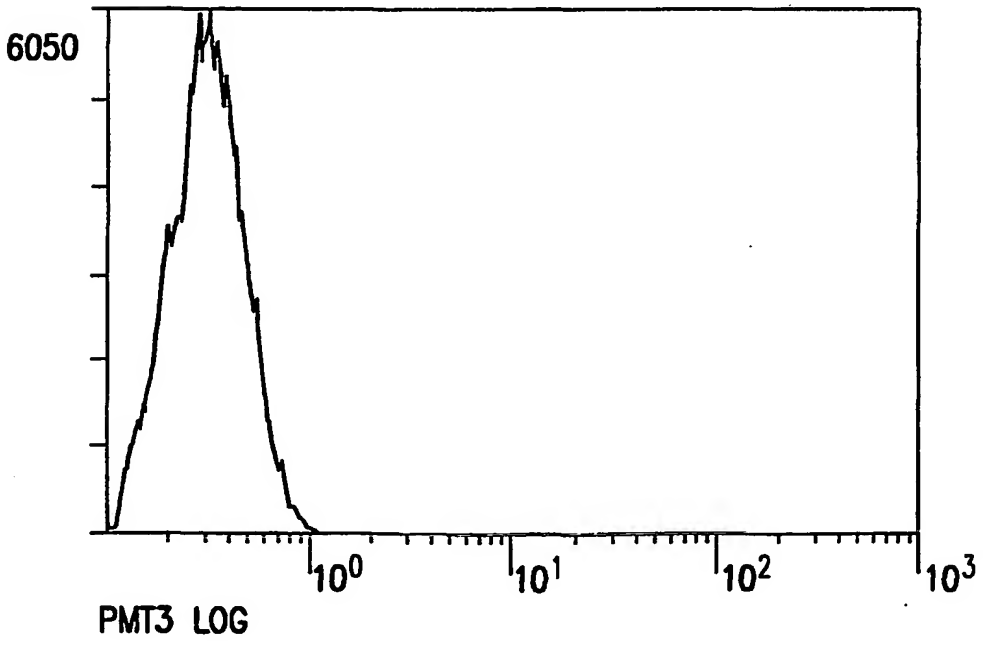

FIG.17A4

GATE:- UNGATED

FILENAME:- Olaf_cntrol_8-11-00.LMD

MEAN CALCULATION METHOD:- LOG-LOG

REGION	NUMBER	%TOTAL	%GATED	X-MEDIAN
A	502458	76.28	76.28	373.0
B1	99	0.02	0.02	0.3
B2	1	0.00	0.00	3.7
B3	658574	99.98	99.81	0.3
B4	1095	0.17	0.17	5.2
D	174	0.03	0.03	13.7

FIG.17A5

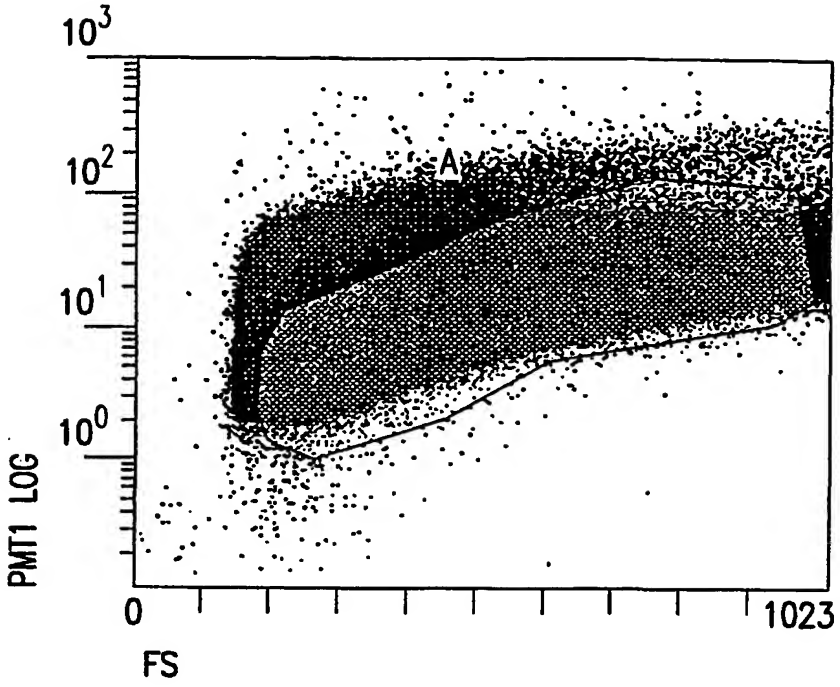

FIG.17B1

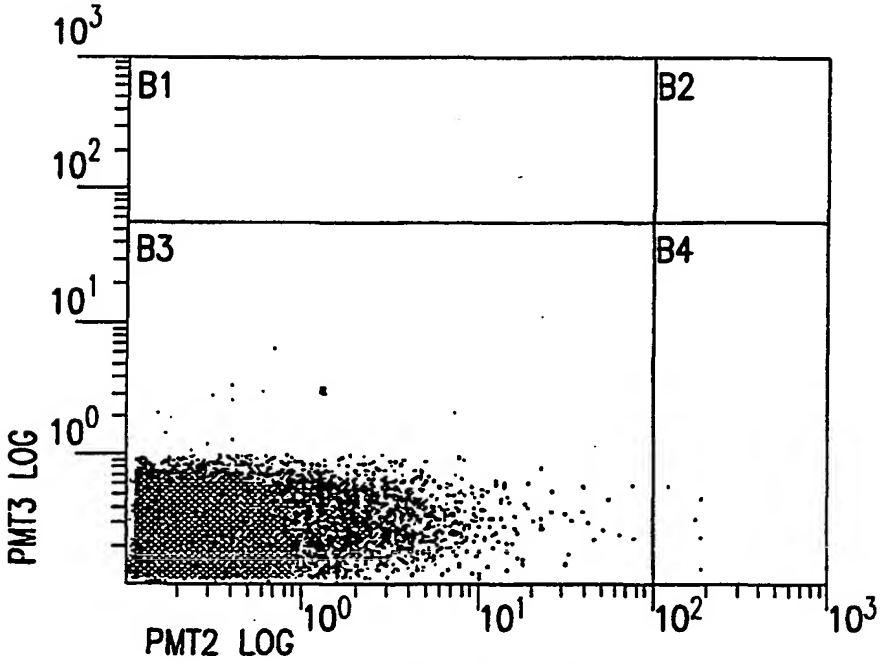

FIG.17B2

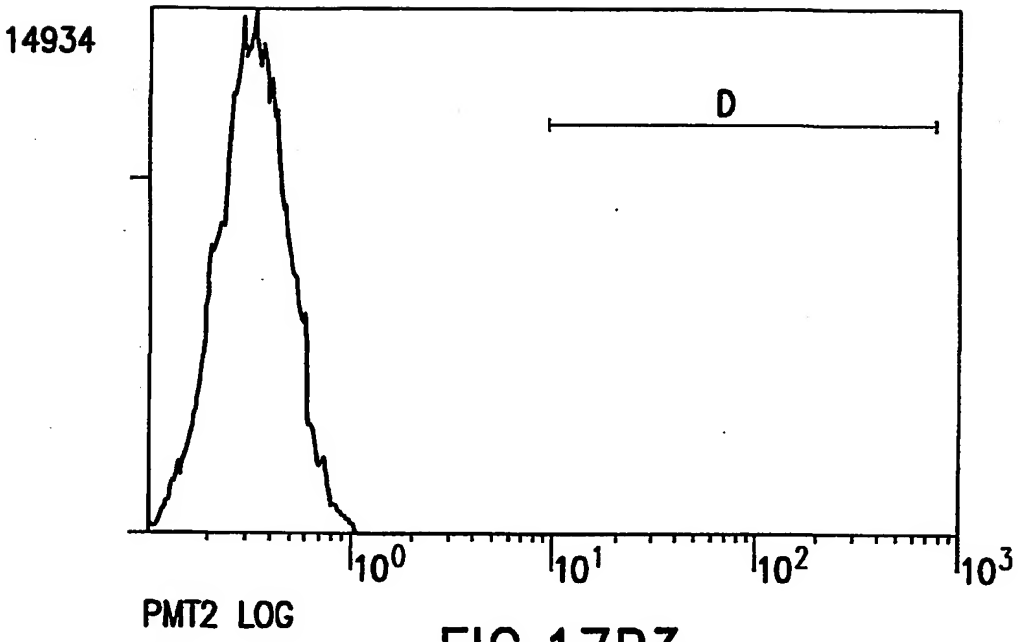

FIG.17B3

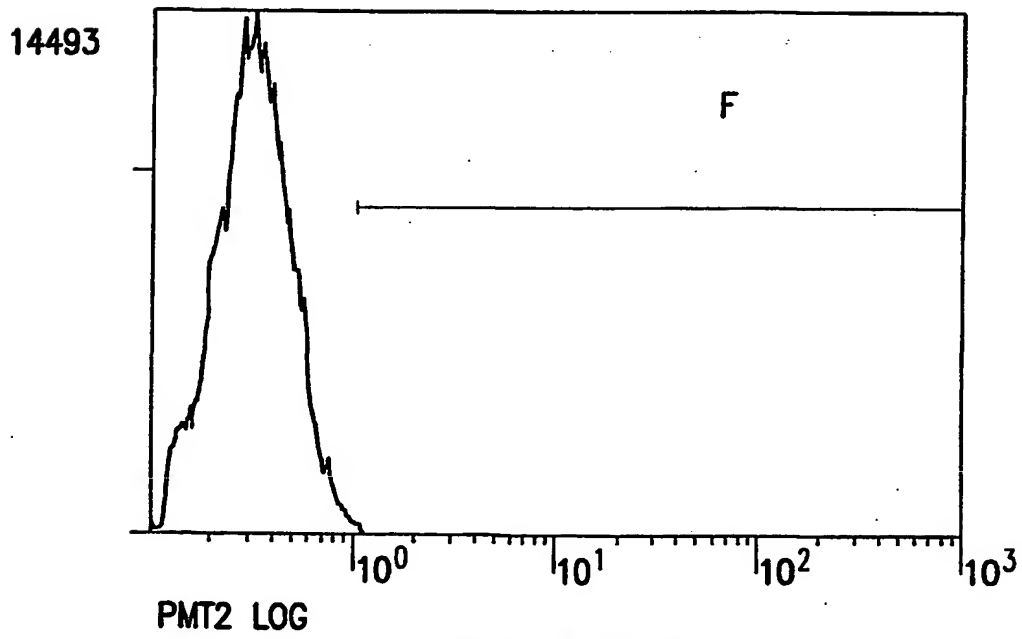

FIG.17B4

GATE:- UNGATED

FILENAME:- 00001722 567.LMD

MEAN CALCULATION METHOD:-LOG-LOG

REGION	NUMBER	%TOTAL	%GATED	X-MEDIAN
A	1304963	83.83	83.83	398.0
B1	4	0.00	0.00	0.3
B2	2	0.00	0.00	162.0
B3	1556736	100.00	100.00	0.3
B4	11 —→ 50	0.00	0.00	182.1
C	13	0.00	0.00	180.5
D	179	0.01	0.01	15.2
F	664	0.04	0.04	1.0

FIG.17B5

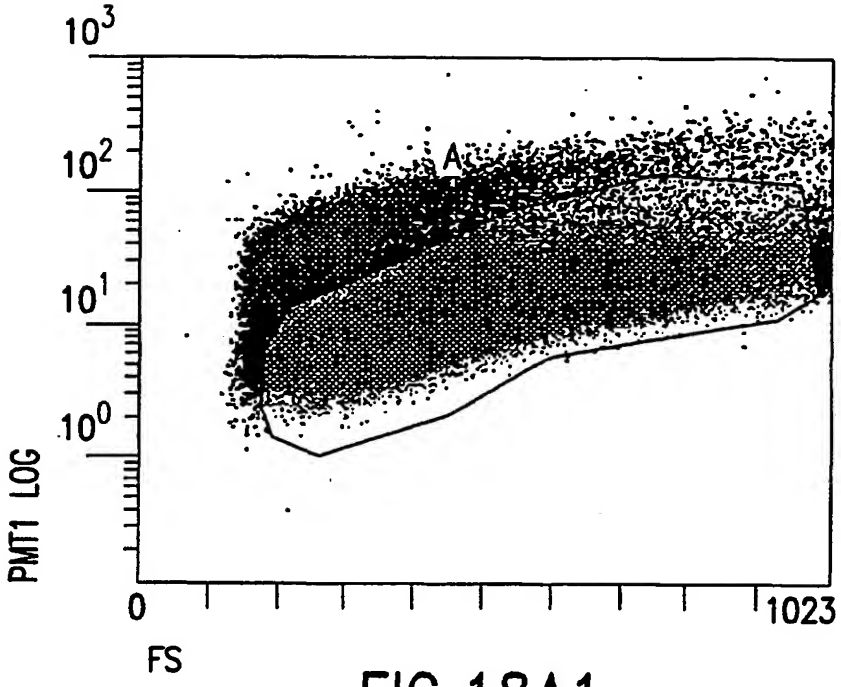

FIG.18A1

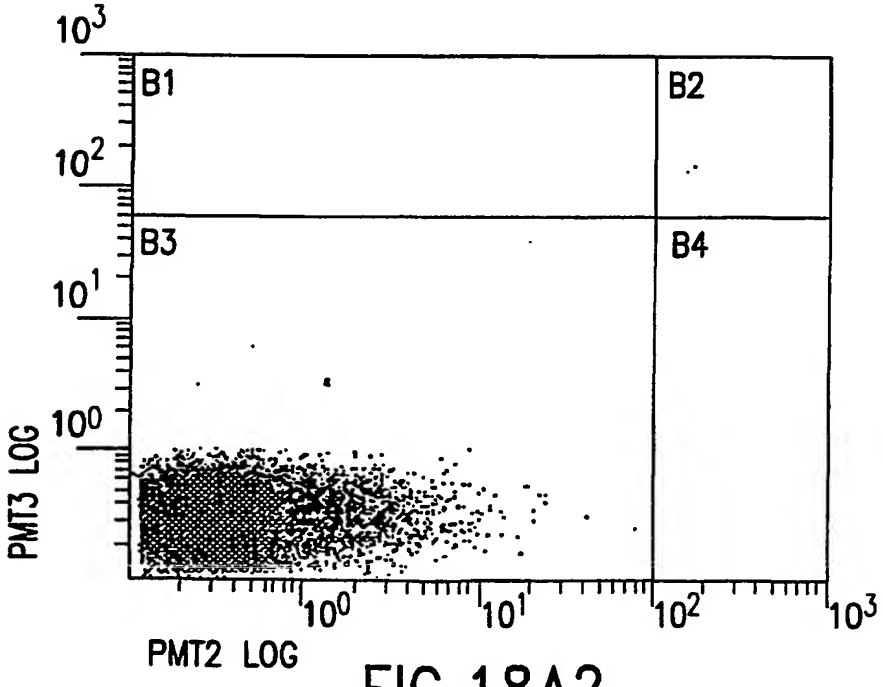

FIG.18A2

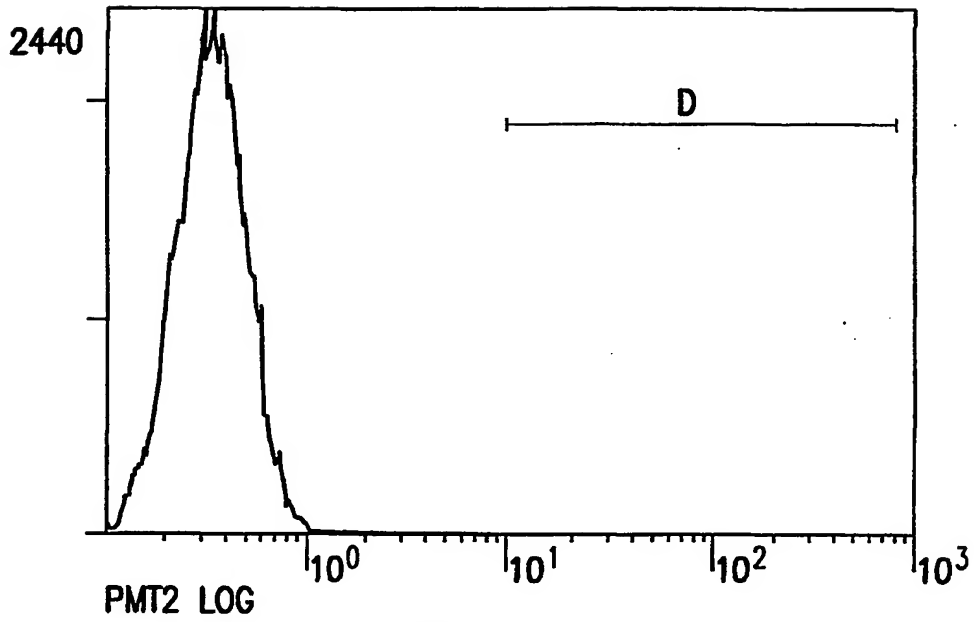

FIG.18A3

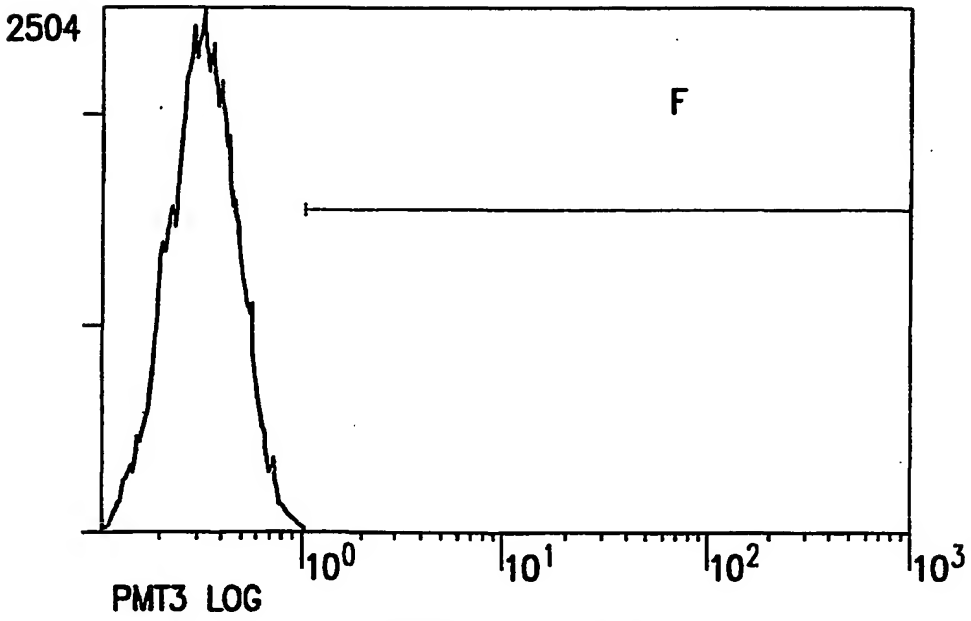

FIG.18A4

GATE:- UNGATED

FILENAME:- 00001720 565.LMD

MEAN CALCULATION METHOD:- LOG-LOG

REGION	NUMBER	%TOTAL	%GATED	X-MEDIAN
A	189157	72.32	72.32	437.0
B1	0	0.00	0.00	0.1
B2	10	0.00	0.00	166.4
B3	261563	100.00	100.00	0.3
B4	0	0.00	0.00	0.1
C	10	0.00	0.00	166.4
D	52	0.02	0.02	15.3
F	121	0.05	0.05	1.0

FIG.18A5

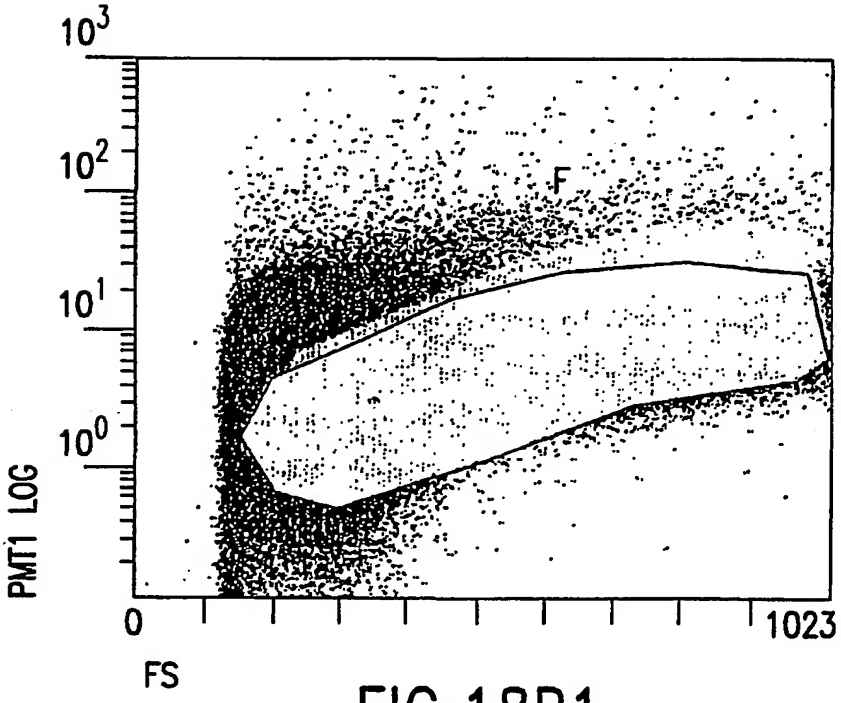

FIG.18B1

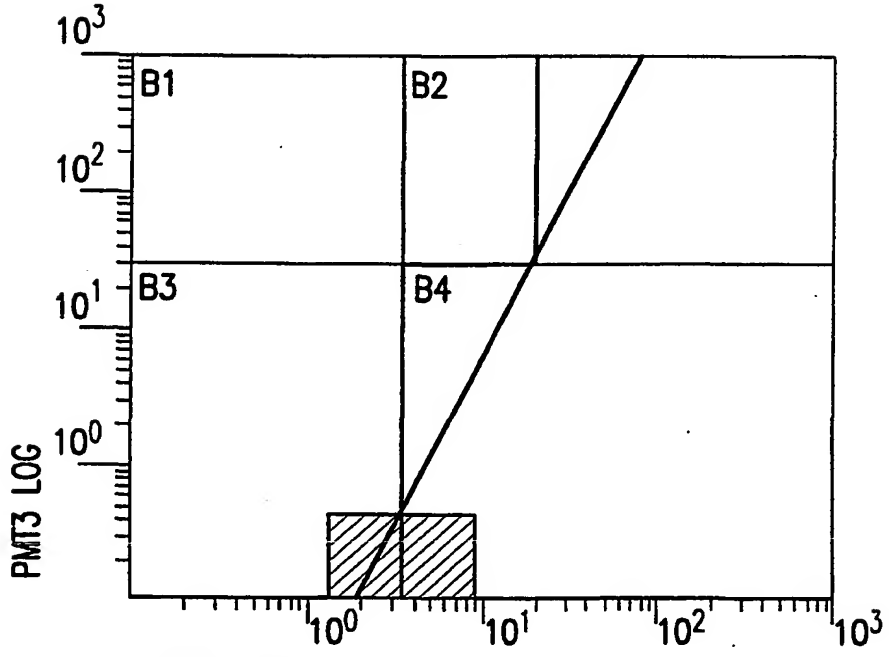

FIG.18B2

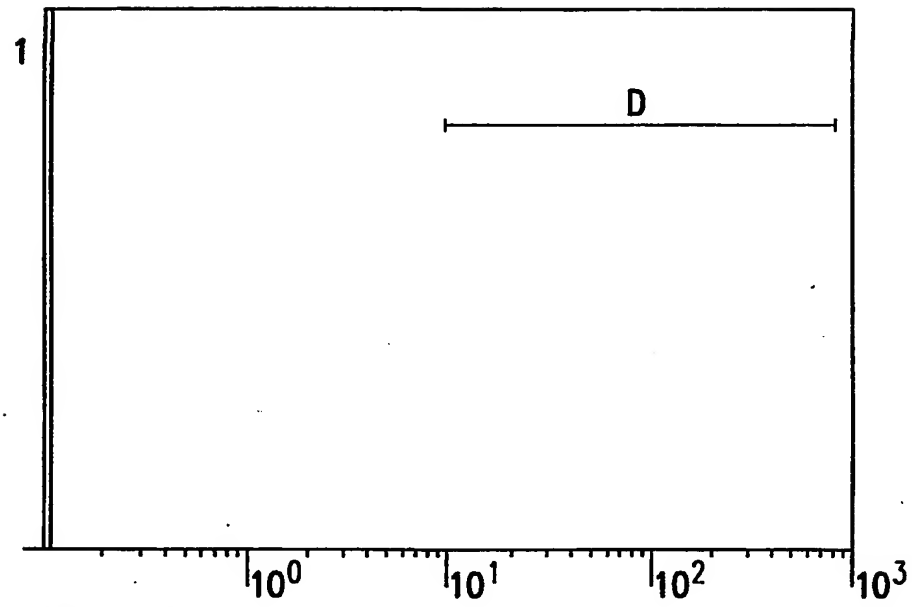

FIG.18B3

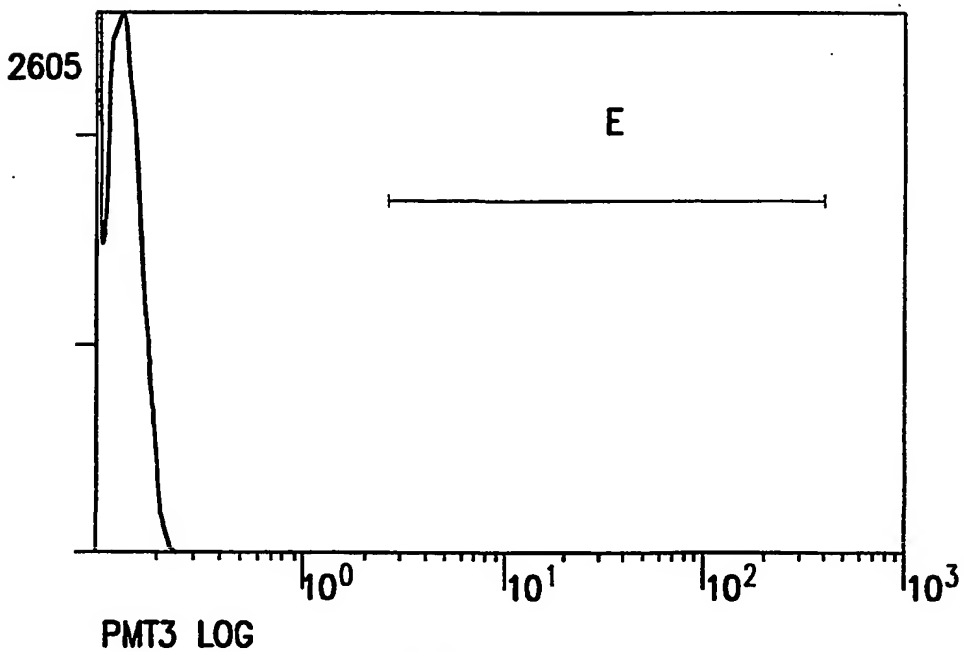

FIG.18B4

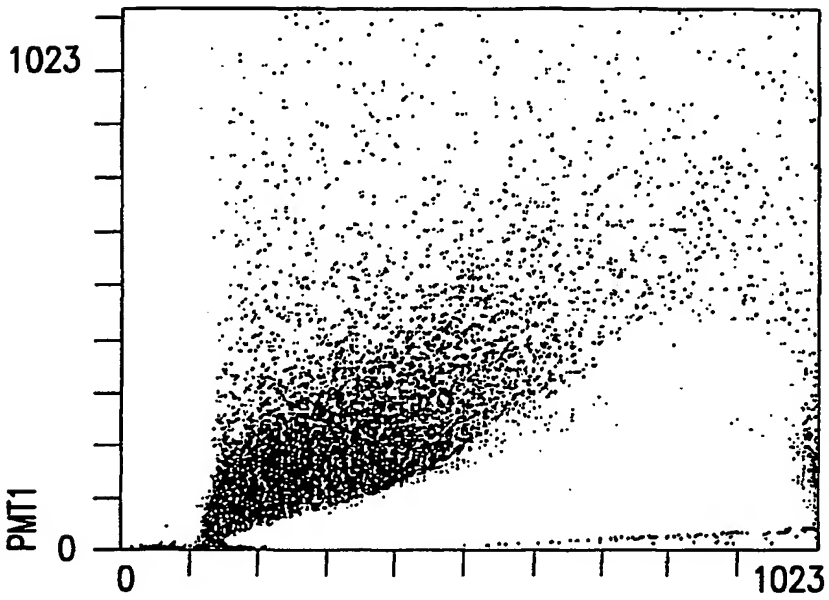

FIG.18B5

GATE:- UNGATED

FILENAME:- 00001225 119.LMD

MEAN CALCULATION METHOD:- LOG-LOG

REGION	NUMBER	%TOTAL	%GATED	X-MEDIAN
B1	2	0.00	0.00	0.3
B2	0	0.00	0.00	0.1
B3	1386992	100.00	100.00	0.1
B4	6	0.00	0.00	4.2
C	6	0.00	0.00	4.2
D	0	0.00	0.00	0.1
E	230	0.02	0.02	4.9
F	1072122	77.30	77.30	429.0

FIG.18B6

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(26) Publication Language: English

(30) Priority Data:
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(72) Inventors: **BEAUDET, Arthur**; 48 Stillforest Drive, Houston, TX 77024 (US). **BODAMER, Olaf**; Waehringer Guertel 18-20, A-1090 Vienna (AT). **KILLARY, Ann**; 4109 Southwestern Street, Houston, TX 77005 (US). **LOVELL, Mercedes**; 10710 Glenfield Ct., Houston, TX 77096 (US).

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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15 May 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/029107 A3

(54) Title: METHOD OF DETECTION AND INTERPRETATION OF MUTATIONS THROUGH EXPRESSION OR FUNCTION TESTS OF HAPLOID GENES

(57) Abstract: The present invention relates to a method for detection and interpretation of loss-of-function or gain-of-function mutations for test genes of interest. The genes of interest include those associated with inherited genetic disorders. The present invention involves the process of obtaining a sample of genetic material from an individual in the form of tissue or cells, separation of the genetic material from the cells of the individuals into haploid sets by transferring the individual chromosomal entities into a population of target cells, and monitoring the target cell population for successful transfer and expression of the test genes of interest using various functional, immunological and structural assays.

INTERNATIONAL SEARCH REPORT

Internati· ·pplication No
PCT/Us 01/30965

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G01N33/50 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAN HAI ET AL: "Conversion of diploidy to haploidy" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 403, no. 6771, 17 February 2000 (2000-02-17), pages 723-724, XP002189209 ISSN: 0028-0836 the whole document ---	1-49
P,X	WO 01 27324 A (KINZLER KENNETH W ;PAPADOPOULOS NICHOLAS (US); YAN HAI (US); VOGEL) 19 April 2001 (2001-04-19) the whole document ---	1-49
Y	US 4 806 476 A (AVNER BARRY ET AL) 21 February 1989 (1989-02-21) the whole document ---	1-49
	-/--	

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Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2003	27/02/2003

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Internatic Application No
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